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UNIVERSITY..... University of Alberta.....

DEGREE FOR WHICH THESIS WAS PRESENTED..... M. Sc. .....

YEAR THIS DEGREE GRANTED..... Fall 1974.....

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DATED..... October 4 1974

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THE UNIVERSITY OF ALBERTA

EFFECT OF UROPHYSECTOMY AND PREOPTIC NUCLEUS LESIONING ON IONIC AND OSMOTIC REGULATION IN THE GOLDFISH (Carassius auratus)

by

© MARY-JANE TURTLE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

FALL, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Effect of Urophysectomy and Preoptic Nucleus Lesioning on Ionic and Osmotic Regulation in the Goldfish (Carassius auratus) submitted by Mary-Jane Turtle in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The effects of urophysectomy and preoptic nucleus lesioning on the osmotic and ionic regulation of Carassius auratus L. were investigated.

Goldfish were maintained at 20°C and ambient photoperiod throughout the study. An experimental group consisted of urophysectomized, preoptic nucleus lesioned or combined preoptic nucleus lesion/urophysectomized fish and their respective sham-operated and intact control fish.

Blood samples were taken at postoperative time periods of five, ten and twenty days and plasma Na^+, Cl^- and Ca^{++} concentrations determined. Urine Na^+, Cl^- and Ca^{++} concentration and urine flow were measured at five and ten day postoperative recovery times on preoptic nucleus lesioned and urophysectomized fish only.

Urophysectomy caused a reduction in plasma Na^+ concentration and urine flow at five days postoperatively. Urine Na^+, Ca^{++} and Cl^- excretion rates were reduced at five days following urophysectomy as a result of the reduction in urine flow in these fish. Plasma Na^+ and urine flow had returned to near normal control values by ten days. Urophysectomy did not alter plasma Ca^{++} or Cl^- levels, or urine osmolality, Na^+, Ca^{++}, Cl^- concentrations

at any of the postoperative times.

Lesioning of the preoptic nucleus caused reduction in plasma Na^+ concentration at five and ten days postlesion. Plasma Ca^{++} and Cl^- were not changed at any postoperative time period. Urine Na^+, Ca^{++} and Cl^- levels were increased at both the five and ten day sampling periods, whereas urine flow was decreased at both of these sampling times.

The reduction in urine flow compensated for the increase in urine Na^+, Ca^{++} and Cl^- concentrations, hence electrolyte excretion was not altered in the five day lesioned fish. However, at the ten day postoperative time period the electrolyte excretion rates were increased. Incompletely lesioned fish did not show a reduction in plasma Na^+ level at any postoperative sampling time.

Simultaneous removal of the preoptic nucleus and the urophysis resulted in a decrease in plasma Na^+ level equivalent to that produced by either operation alone. Plasma Ca^{++} and Cl^- concentrations were not changed by the combined operation.

The results indicate that both the urophysial and the neurohypophysial peptides have a diuretic effect on the teleost kidney. This diuresis is most likely due to changes in GFR. In addition, there appears to be an in-

crease in tubular reabsorption of water in the preoptic nucleus lesioned fish. Plasma Na^+ balance is also affected by both the urophysis and the neurohypophysis, as removal of either of these glands caused hyponatria. However, the mechanisms by which this hyponatremia was produced was different between urophysectomy and preoptic nucleus lesioning. In the urophysectomized fish, the gill was probably the major site of Na^+ loss, while in the preoptic nucleus lesioned fish, the kidney probably played a more important role in Na^+ loss.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the following:

Dr. R. E. Peter, for his support and supervision throughout this study, for his time spent in performing the stereotaxic lesion, and for his assistance in the preparation of the manuscript;

Dr. W. C. Mackay, for his guidance and for his critical review of the manuscript;

Dr. R. J. Christopherson, for his review of the manuscript;

Dr. A. Neil Cuthbertson, for his assistance in the development of a technique for urophysectomizing fish;

V. E. Gill, for her help in sectioning the brain tissue;

My fellow graduate students who provided many stimulating ideas and moral support;

The University of Alberta and the Department of Zoology who provided financial support in the form of graduate teaching assistantships and intersession bursuries.

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INTRODUCTION

The relationship of the caudal neurosectory cells (the Dahlgren cells) and the urophysis (the neurohemal organ) forming a neurosecretory system was first described by Enami (1955). Since this time a caudal neurosecretory system has been described in all teleosts, elasmobranchs, holosteans and chondrosteans investigated (Fridberg, 1962; Fridberg and Bern, 1968; Bern, 1969) and it has been the subject of numerous histological, morphological and pharmacological studies (see Bern et al., 1967; Bern, 1967; Bern, 1969; Lederis et al., 1970; Berlind, 1973).

There is considerable evidence that the urophysis plays a role in the hydromineral balance of teleost fish (see review Bern, 1969; Berlind, 1973). Although urophysial extracts have been shown to produce diuresis, increases in glomerular filtration rate, changes in branchial Na^+ flux and elevation in blood pressure in various species of teleosts (Maetz et al., 1964; Bern et al., 1967; Chan et al., 1969; and Chester Jones et al., 1967, 1969b); urophysectomy has failed to produce any effects on osmoregulation (Takasugi and Bern, 1962; Chester Jones et al., 1969; Berlind, 1973). Thus, the emphasis of previous in-

vestigations has been on the chemical identities and the pharmacological properties of the urophysial factors (Lederis, 1973). Their physiological activities have, in the past, been largely ignored (Berlind, 1973).

The lack of a common reference preparation has caused confusion in regard to the physiological role and the number and nature of active principles which may occur in the urophysis (Bern and Lederis, 1969). To date, at least four urophysial factors (Urotensins) have been identified (Berlind, 1973). Urotensin I consists of two rat hypotensive components which have long and short term effects and are separable by chromotography (Zelnik and Lederis, 1973). Preliminary observations suggest that in eels Urotensin I has pressor activity similar to but not as potent as Urotensin II, and that it also causes a decrease in glomerular filtration rate (Lederis, 1973). Urotensin II causes an elevation in eel blood pressure (Chan et al., 1969; Zelnik and Lederis, 1973) and increases the frequency of contractions of trout bladder, mudsucker intestine, guppy oviduct (Lederis, 1970a, b, c) and the mudsucker sperm duct (Berlind, 1972). A preliminary report indicates that Urotensin II also causes an increase in glomerular filtration rate in eels (Lederis, 1973). Urotensin III stimulates branchial Na^+ influx in goldfish (Maetz et al., 1964a). Urotensin IV, the hydrosmotic factor, has been demonstrated by Lacanilao (1972a, b) to

probably be arginine vasotocin (AVT).

In teleosts there are two octapeptides in the neural lobe of the pituitary, AVT and isotocin (4 Ser - 8 Ile - oxytocin) (Heller and Pickering, 1961; Sawyer, 1966; Perks, 1969). The cell bodies of the secretory neurons whose axons form the neurohypophysis are located in the nucleus preopticus (NPO) situated in the hypothalmus on either side of the preoptic recess just posterior to the anterior commissure (see Perks, 1969).

AVT has been shown to cause diuresis and increased glomerular filtration rate (GFR) and increased paraminohippuric acid (PAH) clearance in freshwater teleosts (Carassius auratus: Maetz, 1963; Maetz et al., 1964b; Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970; Salmo gairdneri: Holmes and McBean, 1963; Amia and Protopterus aethiopicus: Sawyer, 1966, 1970, 1972, 1973). However, AVT does not cause diuresis in the aglomerular kidney of the marine teleost Opsanus tau even though it does have a pressor effect (Lahlouh et al., 1969a). Although antidiuretic effects of AVT on the teleost kidney have been reported (Salmo gairdneri, Holmes, 1961; Salvelinus namaycush, Hammond, 1969) most of the attention has been focused on the strong diuretic response which follows injections of AVT. At the gill isotocin stimulates the influx of Na^+ while AVT enhances both Na^+ influx and efflux in freshwater

teleosts (Maetz, 1963; Maetz et al., 1964b). In the seawater adapted flounder, Platichthys flesus, oxytocin will stimulate branchial efflux of Na^+, while in freshwater it stimulates influx of Na^+ (Motais and Maetz, 1964). Oxytocin and isotocin will also cause increases in urine flow and inulin clearance in teleosts (Maetz and Julien, 1961; Maetz et al., 1964b; Butler, 1966; Sawyer, 1966; Chester Jones et al., 1969).

There have been no studies on the effects of lesioning of the NPO on osmotic or ionic regulation of Carassius auratus L.. Chan (1969) has electro-cauterized the preoptic area in Anguilla anguilla and measured plasma Na^+, Ca^{++} and PO_4 composition. The sham operation, however, consisted of the removal of the forebrain. The lesioning technique was not decribed nor was the extent of lesion reported. Thus the study cannot be regarded as conclusive in this respect.

With the exception of the aforementioned study, physiological studies on the role of the teleost neurohypophysis in osmotic or ionic regulation have been limited to total or partial hypophysectomy. Hypophysectomy removes both the neurohypophysial and adenohypophysial peptides, thus normal hormonal balance is severely disrupted. And as there are several hormonal systems, such as the adrenocorticoids, prolactin and AVT, believed to

be involved in ionic and osmotic regulation (Olivereau and Ball, 1970), it is difficult to ascertain which system is causing the observed effect. Also, there is considerable evidence that hypophysectomy does not cause the NPO to cease functioning. Instead, there can be a regeneration of the neurosecretory axons and the infundibular stalk forming a "neurohypophysis-like" organ (Sathyanesan, 1966, 1969; Belsare, 1970). When regeneration of the stalk is obvious the neurosecretory neurons appear normal thus suggesting that some of the neurohypophysial functions could be maintained (Sathyanesan, 1970). Therefore, ablation of the NPO, the source of the neurohypophysical peptides, is a more desirable approach to the study of neurohypophysial function.

Investigations of the physiological activities of the urophysial and neurohypophysial peptides in teleosts have largely been confined to replacement therapy and the study of the immediate effects of such treatments. There has been no attempt to relate changes in kidney function with passage of time following preoptic nucleus lesioning or urophysectomy. Although there is evidence that the urophysis as well as the neurohypophysis secrete AVT (see above), there have been no physiological studies where the effect of simultaneous removal of these organs has been examined.

Thus, the objectives of the present study were,

firstly, to determine what effect, if any, ablation of the preoptic nucleus (NPO) and/or urophysectomy have on plasma and urine Na^{+}, Ca^{++} and Cl^{-} concentration and on urine flow in the freshwater, stenohaline teleost Carassius auratus L.. The second objective was to determine if there are any compensatory changes in the osmoregularity capacity in these fishes with respect to postoperative recovery time.

MATERIALS AND METHODS

Care of Study Animals

Mature goldfish, Carassius auratus L. (common and comet varieties) were commercially obtained from Grassyforks Fisheries Co. (Martinsville, Indiana), and shipped by air to the University of Alberta. Upon arrival, the fish were held in dechlorinated Edmonton tapwater at 20°C in large flow-through holding tanks (15391) in the main aquatic facilities of the Department of Zoology. The fish were fed to excess daily with commercial fish food (5/32 pellets, Silver Cup Fish Feed, Ferguson Feeds Ltd., Drinkwater, Saskatchewan).

Two weeks prior to experimentation randomly selected fish were moved to a private research room where they were divided into groups and placed into smaller (1361) continuous-flow holding tanks at 20°C. Operated fish and their respective sham and intact controls were kept in the same holding tank. The light regime followed the ambient photoperiod throughout the study. The fish were fed to satiation twice daily. However, fish used in the renal excretion studies were not fed while catheterized. Postoperative maintenance was as described for

preoperative care.

Experimental Protocol

Three major experimental groups were established; urophysectomized fish, preoptic nucleus lesioned fish and the combined operation of preoptic nucleus lesion/urophysectomy. The above experimental groups were each used for the plasma composition and renal excretion studies, with the exception that the combined preoptic nucleus lesion/urophysectomized fish were used in only the plasma electrolyte study. Plasma composition was measured at the postoperative time periods of five, ten and twenty days, while renal excretion was measured at only five and ten days postoperatively. Included in each experimental group were sham-operated and intact control fish.

OPERATIVE PROCEDURES

Anesthesia

Prior to operative procedures, the fish were anesthetized by immersion in a 0.1 percent solution of tricaine methanesulphonate (Kent Laboratories Ltd., Vancouver, B. C.) in dechlorinated tapwater. Anesthesia was to the point where the fish had lost righting ability and opercular movement was barely detectable. Following anesthetization the fish were weighed, wrapped in damp paper towelling to

prevent desiccation and marked by fin clipping. The operation was then performed (see below). In most cases, the fish recovered immediately from the anesthetic when placed in their holding tanks following the operative procedures. Fish that did not immediately recover were revived by perfusing the gills with oxygenated water. It was necessary during the urophysectomy and the double operation (urophysectomy and preoptic nucleus lesion) to alternately perfuse the gills with a 0.033 percent solution of tricaine methanesulphonate and oxygenated dechlorinated tapwater to maintain anesthesia.

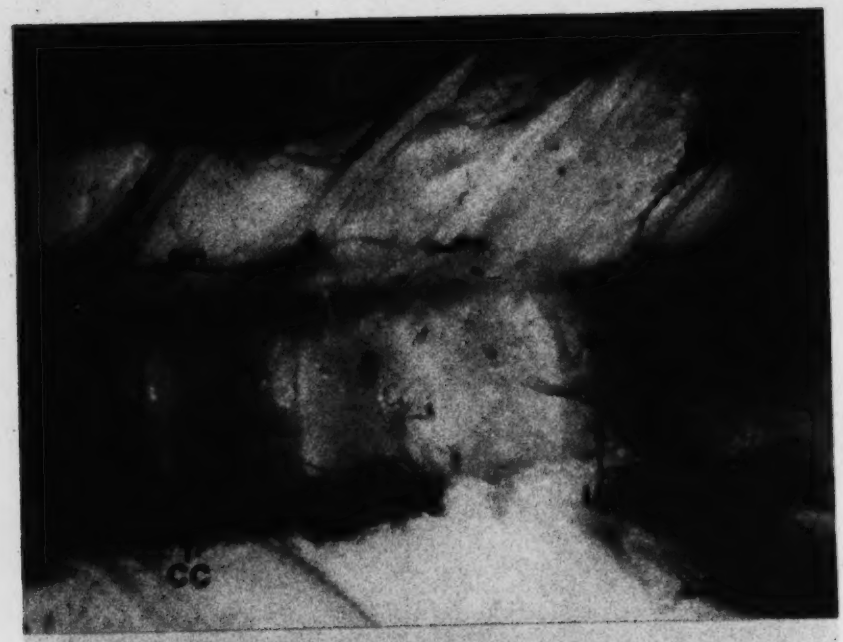
Urophysectomy

In the goldfish, the urophysis is located in a depression on the dorsal surface of the urostyle, the last caudal vertebra, and is surrounded by bone on three sides. The entire urostyle is heavily covered with connective tissue and its ventral surface lies adjacent to the caudal circulatory system (Figure 1a). To accomplish urophysectomy, as described below, it was necessary to dissect out the entire urostyle containing the urophysis and filament terminale (Figure 1b).

An anesthetized fish with only the tail region exposed was placed on its left side on moist paper towelling. The initial incision was made with a sterile no. 15 scalpel blade about 3 mm dorsal to the lateral line in the

FIGURE 1A A dissection of the peduncle region of the goldfish showing the urophysis (UH) in the last vertebral element, the urostyle (US). The spinal cord (SC) was cut at the level of the second last vertebral disc. The caudal circulation (CC) was left intact.

FIGURE 1B The peduncle region of the goldfish after removal of the urophysis. The dotted line bisects the last vertebral disc, and outlines the tissue that was removed during urophysectomy.



1a

1b

caudal peduncle area. This longitudinal incision was from 2.0 to 2.5 cm in length in fish over 100 grams and approximately 1.5 cm in length in fish between 45-100 grams. The incision was made 3-5 mm in depth in order to expose the lateral surface of the caudal vertebrae. The wound was held open with four sterile stainless steel insect pins bent to form retractors. The connective tissue was scraped away from all facets of the urostyle and the last vertebral disc with the edge of the scalpel blade, exposing the urostyle in its entireity. The spinal cord was then cut at the level of the second last vertebral disc. A pentagonal section was made around the urostyle with the base of the pentagon bissecting the last vertebral disc. The urostyle with the urophysis intact and the spinal cord fragment were then carefully removed with forceps.

The wound was stitched using a ½ inch half-circle reverse cutting atraumatic needle with an attached 5-0 silk suture (Opthalmic suture, Davis and Geck, Division of American Cyanamid Company, Danbury, Connecticut). Two hemostatic stitches were made to reduce hemorrhage and three to four skin stitches were used to finish closing the wound. Barring complications, such as excessive bleeding, the time required to complete the operation was 12 to 15 minutes. Fish used in the plasma composition study were between 45-75 grams while those used in the renal study were between

110-165 grams.

The same procedure was followed for the sham operation. However, in this case, neither the spinal cord nor the vertebral disc were severed, nor was the urophysis (urostyle) removed.

Preoptic Nucleus Lesion

The operative procedures for electrolytically lesioning the NPO were as described by Peter (1970), and as modified by Peter and Gill (1974). The direct current anodal lesions were made by passing 1 mA of current for 20 seconds. The electrodes were no. 00 stainless steel insect pins insulated with Insl-X (Insl-X Products Corp., Yonkers, New York), as described by Peter (1970). The coordinates for electrode placement were +0.9,M,D 2.0 (Peter and Gill, 1974).

Sterile gut (Davis and Geck) was used to seal the skull cap on the ten and twenty day experimental animals, while silk suture (3-0) was used on the five day experimental fish. Fish used in the plasma composition studies were between 35 and 60 grams, while those used for the renal study were larger, between 60 and 90 grams, due to the problem of catheterizing the urinary duct of the smaller fish. The sham-operated animals underwent the same surgical procedures, with the exception that no

FIGURE 2. Cross section through the mid nucleus preopticus (NPO) region of a control animal. The NPO cells are intensely stained with paraldehyde fuschin. Nucleus endopeduncularis, NE; optic tract, OT; preoptic recess of the III ventricle, PR; telencephalon, T.

FIGURE 3. Cross section through the mid nucleus preopticus region of a partially (incompletely) lesioned goldfish. There is one stainable neurosecretory cell stainable for neurosection remaining in the section (arrow).

2

3

FIGURE 4. Cross section through the mid nucleus preopticus region of a five day completely lesioned goldfish. The lesioned area is outlined with arrows. No stainable neurosecretory cells remain.

FIGURE 5. Cross section through the mid nucleus preopticus region of a ten day completely lesioned goldfish. The lesioned area is outlined with arrows. No stainable neurosecretory cells remain.

FIGURE 6. Cross section through the mid nucleus preopticus region of a twenty day completely lesioned goldfish. The lesioned area is outlined with arrows. No stainable neurosecretory cells remain.

4

5

6

current was passed through the electrode. To determine whether the lesions were complete serial sections of 8 µm were made of the forebrain of each experimental fish (Figures 2, 3, 4, 5, and 6). The sections were stained with paraldehyde fuchsin and counterstained with fuchsin, ponceau xylidine and fast green. A fish was regarded as partially lesioned (incompletely lesioned) if one or more stainable neurosecretory cells remained in the preoptic area.

Preoptic Nucleus Lesion/Urophysectomy

In the joint operation of preoptic nucleus lesion and urophysectomy, the same surgical procedures were followed as described for the single operation. The fish used in this experiment were between 45 to 75 grams. The sham-operated fish were treated in the same manner as the previously described sham groups.

SAMPLING TECHNIQUES

Plasma Electrolyte Study

The operated fish (urophysectomized, preoptic nucleus lesioned or urophysectomized and preoptic nucleus lesioned) and their respective sham-operated and intact controls were sampled at five, ten and twenty days post-operatively. Individual fish were removed from the hold-

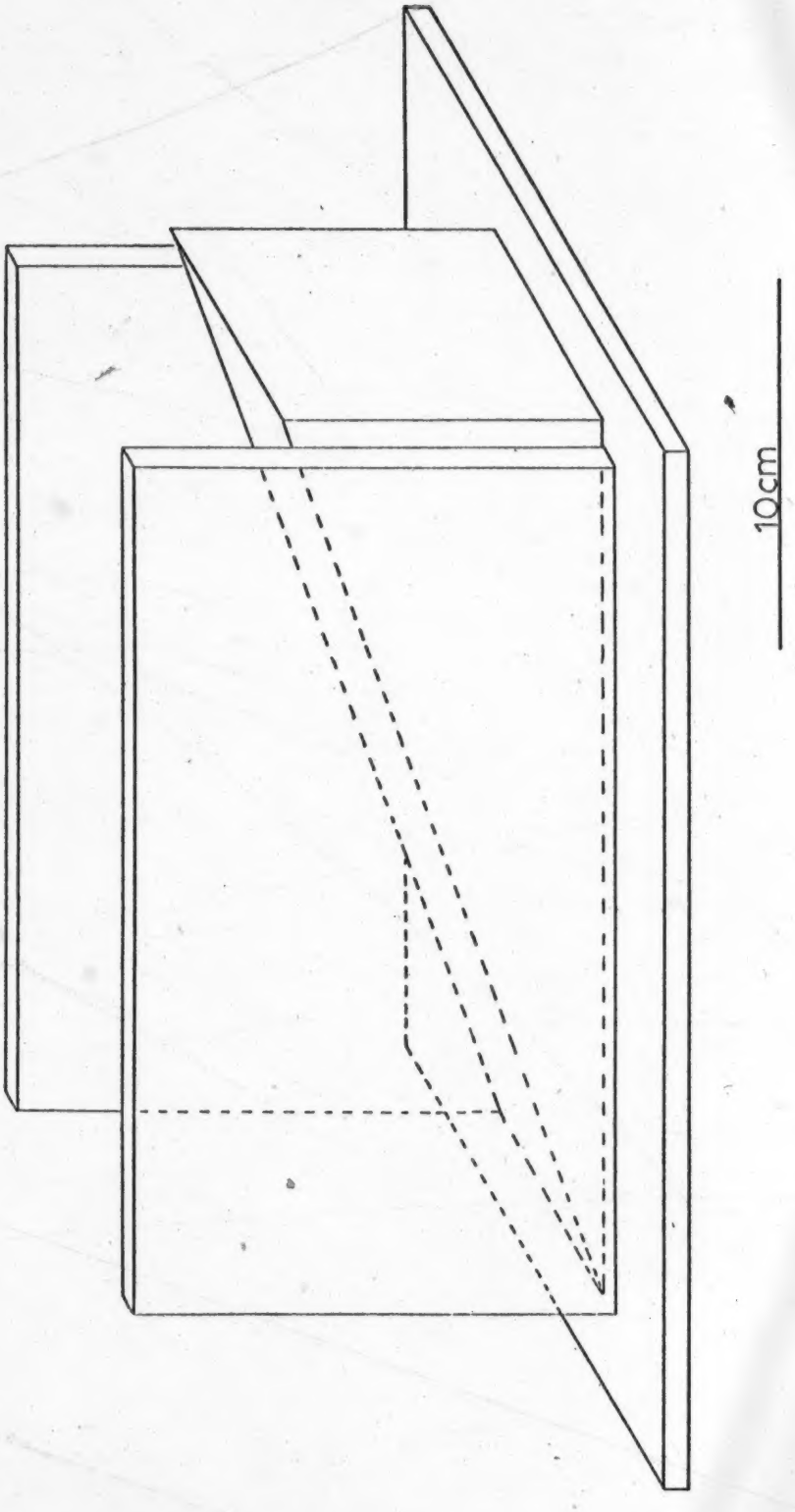
ing tank with minimum disturbance to the other fish in the tank, wrapped in paper towelling and weighed. A blood sample was taken and the fish then terminated.

Blood samples of ½ to 1 cc were obtained by puncture of the caudal circulation using a 1½ inch, 23 guage needle with a heparinized (ammonium heparin) 2½ cc syringe, according to the technique of Mackay (pers. comm.). To avoid hemolysis or coagulation the blood was immediately centrifuged for two minutes. The plasma was then pipetted into a 400 µl centrifuge tube and frozen. The samples were stored at -15°C until analysis, at which time the plasma was analyzed for Na^+, Ca^{++} and Cl^- concentrations (see Analytical Procedures).

Renal Study

Individual fish were removed from the holding tanks and anesthetized with tricaine methanesulphonate. The fish were then weighed and a catheter inserted into the urinary duct so that the tip of the catheter was in the urinary bladder. The operation was carried out in a plexiglass operating box (25 x 13 x 8 cm) that held the fish rigid while exposing only its ventral surface (Figure 7). Urinary catheters were fabricated from lengths of PE 50 and PE 90 tubing (Intramedic, Clay-Adams Inc.). A 5 cm length of PE 90 tubing was moulded to the shape of the ventral body surface

FIGURE 7. The plexiglass operating box used to hold the goldfish while inserting the catheter. An anesthetized fish with its head region wrapped in wet paper towelling was placed on its side on the incline. The tail region was exposed to allow for catheterization.


10cm

of the fish by heating and the sides of the last 3 mm of one end perforated to allow urine to entire from the entire circumference of the tubing (Figure 8). One end of a 60 cm length of PE 50 was inserted inside the unperforated end PE 90 collecting tube to form a tight joint fit. The catheter was long enough to extend from the fish into the collection cylinder. The catheter was made entirely of a 60 cm length of PE 50 tubing when urine was collected from fish under 100 grams.

To prevent leakage of urine after the catheter had been inserted, a purse string suture was placed around the posterior side of the rectum and around the opening of the urinary duct posterior to the bladder, according to the technique of Mackay (pers. comm.). To avoid having the catheter pulled out, it was stitched to both the anal and caudal fins (Figure 8). The entire catheterization was completed in five minutes. The fish was then transferred to an experimental box (25 x 5 x 10 cm) (Figure 9), where it was allowed to recover.

To eliminate the effects of handling diuresis (see Hunn and Willford, 1970), urine was not collected until twenty-four hours after catheterization and the surrounding environment was kept as quiet as possible during the urine collection. Urine was then collected under mineral oil for 24 ± 1 hours in an acid washed 25 cc gra-

FIGURE 8. Urinary system of the goldfish showing the position and construction of the catheter used to collect urine.

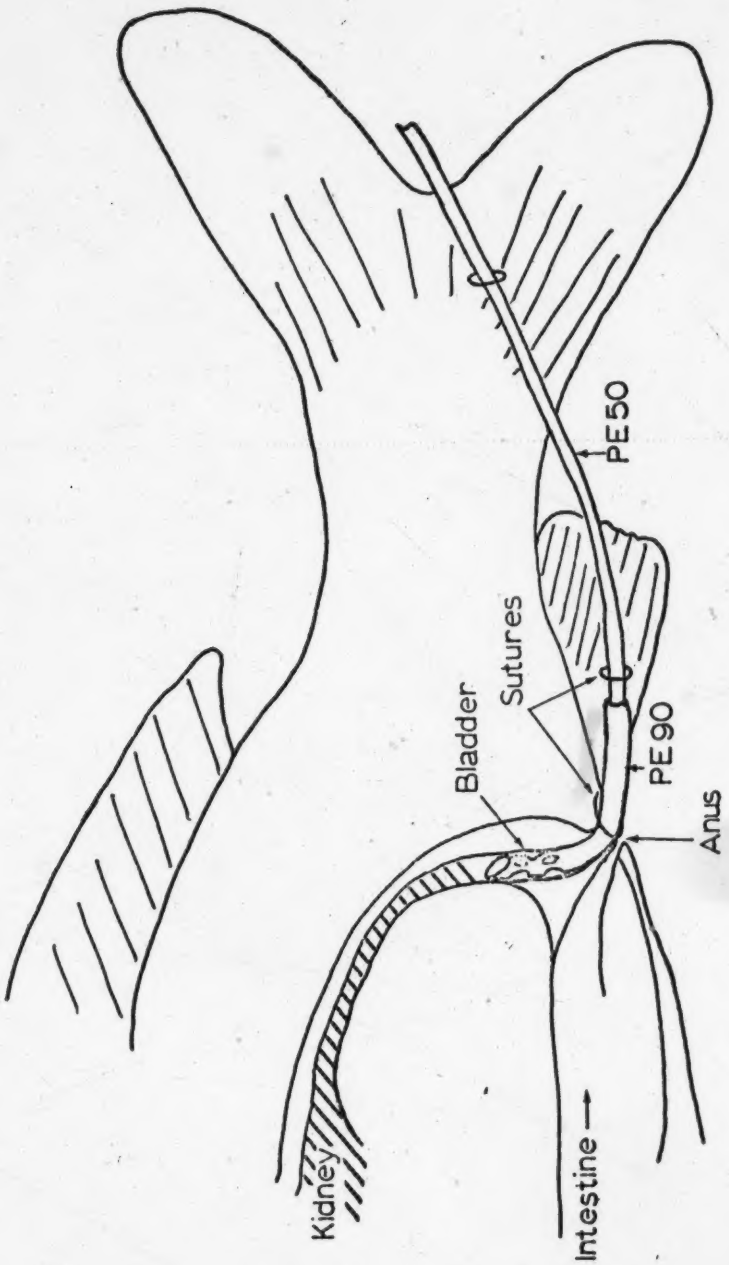
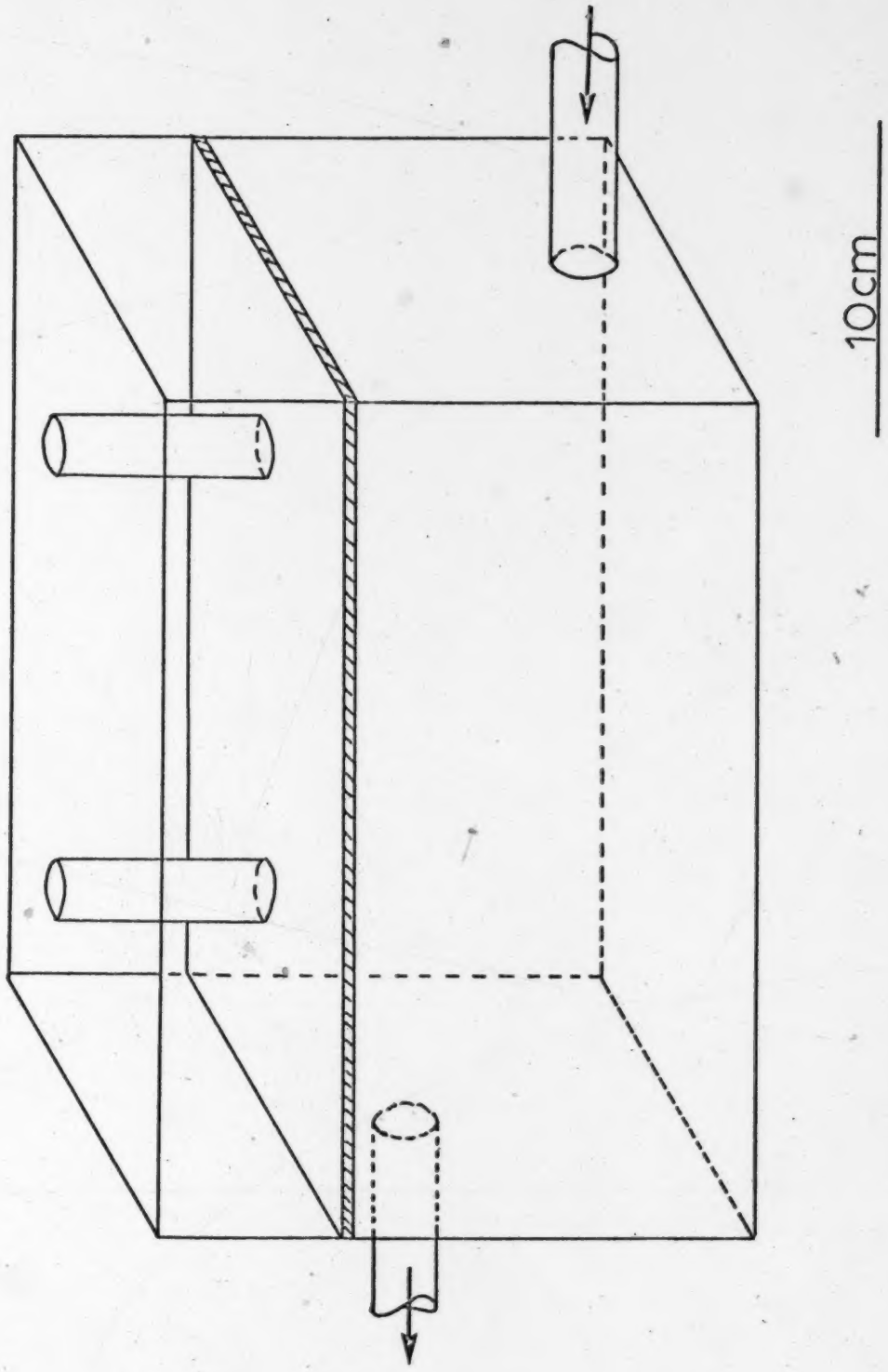

PE50
Sutures
Bladder
PE90
Anus
Kidney
Intestine

FIGURE 9. Experimental chamber used to contain the goldfish during urine collection. The plexiglass box was designed to allow only limited movement so that the urinary catheter could not be pulled out when the fish struggled. Aerated water was pumped to the chamber from the main aquatic facilities supplies, where the temperature was controlled.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duated cylinder located 30 cm below the experimental chamber. The graduated cylinder was covered with parafilm to further reduce evaporative loss during the collection period. At the end of the twenty four hour collection period, total urine volume was measured and the urine was placed in an acid washed 10 cc plastic test tube and immediately frozen. The samples were stored at -15°C until analysis, at which time total osmolality, Na^+, Ca^{++} and Cl^- levels were determined (see Analytical Procedures).

Three experimental boxes were available. Thus, an intact control, a sham-operated and an operated fish were run simultaneously.

ANALYTICAL PROCEDURES

Na^+ and Ca^{++} concentrations in urine and plasma were determined by flame emmission on a Jarrel-Ash Flame emmission-atomic absorption Spectrophotometer (Model 82-270 Atomsorb) using standard grade acetylene (Liquid Air Canada Ltd., Edmonton, Alberta) as fuel. For Ca^{++} determinations a nitrous oxide-acetylene flame was used while an air-acetylene flame was used for Na^+ analysis. Cl^- concentrations were measured by amperometric titrations with silver ions using a Buchler - Cotlove Chloridometer (Model 4-2000). Urine osmolality was determined by freezing point depression using a Fiske Osmometer (Model C-66A). The procedures outlined in the operators manual

for the above analytical instruments were followed.

All chemicals used throughout the study were analytical grade. Glassware was washed with sulphuric acid saturated with potassium dichromate and then stored in double distilled water. Duplicate determinations were made on all samples for each ion. Electrolyte excretion rates were calculated by multiplying urine flow by the urine electrolyte concentration.

STATISTICAL TESTS

The Students' t-test for unpaired samples (Sokal and Rohlof, 1969) was used to determine if there were significant differences between the experimental groups. Differences were considered to be statistically significant when the p value was less than 0.05.

RESULTS

Urophysectomy

Plasma Electrolyte Levels

The sham-operated and intact controls maintained similar plasma Na^+ concentrations throughout the study (Figure 10) (Appendix, Table 1). The plasma Na^+ concentration in the five day urophysectomized animals was significantly lower than in the sham-operated and intact control groups for that postoperative time period (Figure 10) (Appendix, Table 1). The ten and twenty day urophysectomized animals had plasma Na^+ levels that were not significantly different from their respective sham and intact controls (Figure 10) (Appendix, Table 1). The plasma Na^+ concentration was significantly lower in the five day urophysectomized animals compared to the ten and twenty day fish ($p<0.01$ and $p<0.001$ respectively). There was no significant difference between the ten and twenty day animals (Figure 10) (Appendix, Table 1).

The sham-operated animals maintained plasma Cl^- levels which were not significantly different than the intact controls at any of the postoperative times (Figure 11) (Appendix, Table 1). The plasma Cl^- levels of the

FIGURE 10. The effect of urophysectomy on plasma sodium concentration in goldfish at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

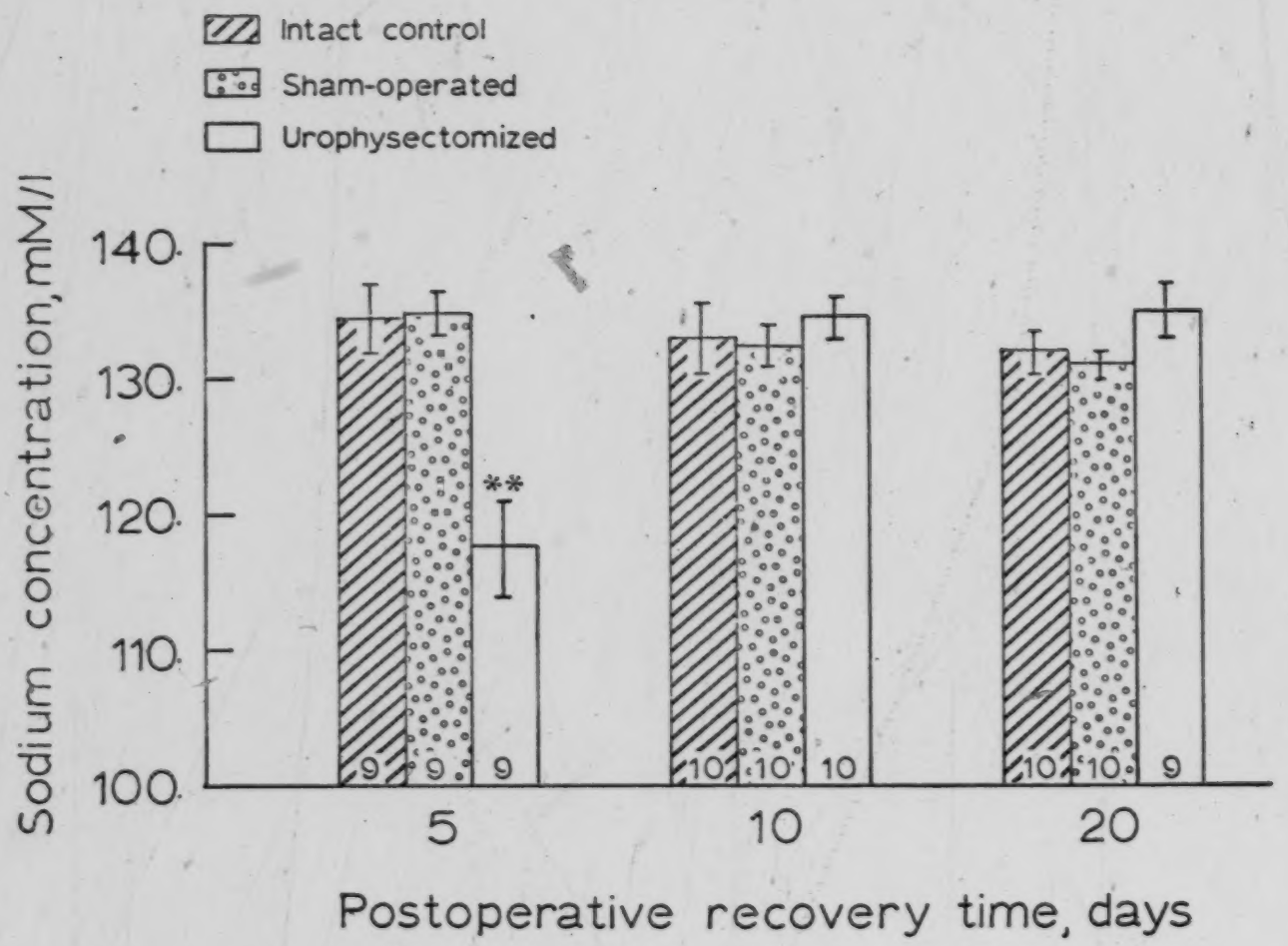
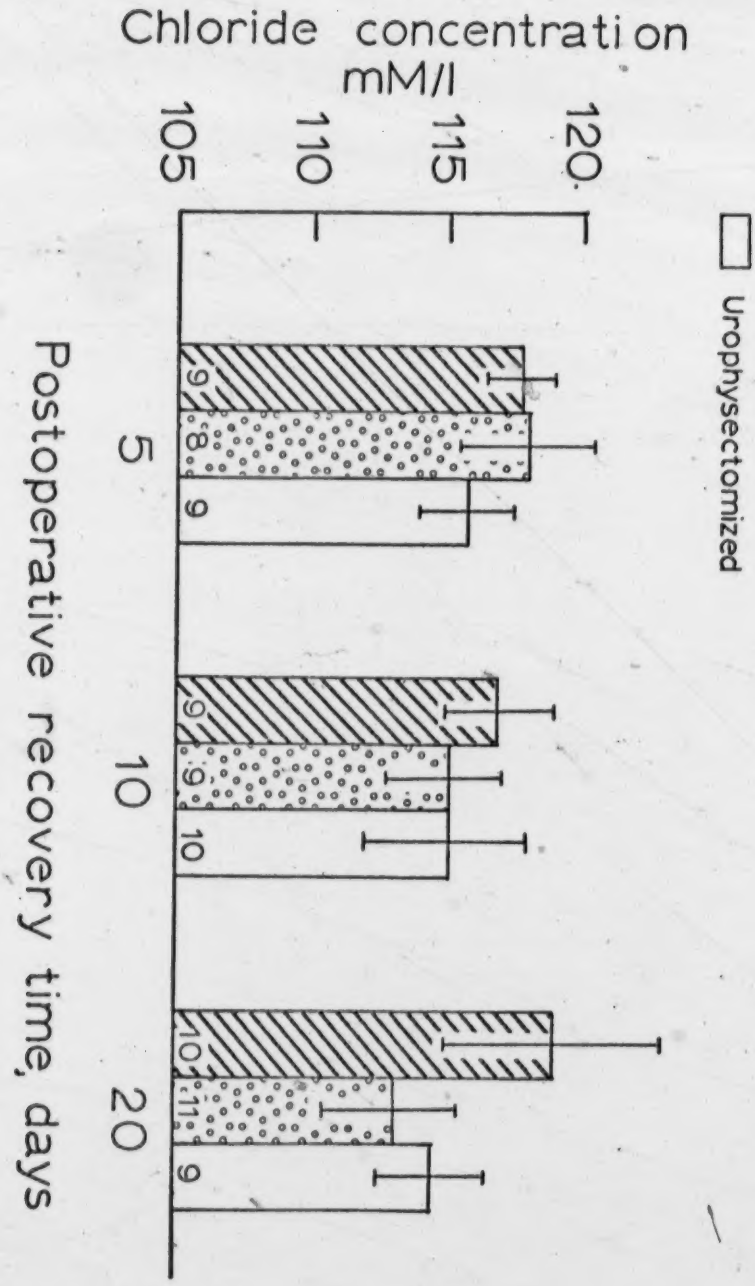

Intact control
Sham-operated
Urophysectomized
Sodium concentration, mM/l
140
130
120
110
100
**
9
9
9
10
10
10
10
10
9
5
10
20
Postoperative recovery time, days

FIGURE 11. The effect of urophysectomy on plasma chloride concentration in goldfish at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.


Chloride concentration
mM/l
105
110
115
120
Intact control
Sham-operated
Urophysectomized
9
8
9
9
9
10
10
11
9
5
10
20
Postoperative recovery time, days

urophysectomized fish did not differ from the sham or intact control groups at any of the postoperative times (Figure 11) (Appendix, Table 1). There also were no significant differences in plasma Cl^- levels between the five, ten and twenty day urophysectomized fish.

Plasma Ca^{++} levels were not changed by urophysectomy (Figure 12) (Appendix, Table 1). The plasma Ca^{++} concentrations of the sham-operated control groups were not significantly different than the intact controls at any of the postoperative times. The five, ten and twenty day urophysectomized fish had plasma Ca^{++} levels that were not significantly different from their respective sham-operated and intact control groups, nor were there any significant differences between these groups.

Renal Study

The effects of urophysectomy on urine flow (V) are shown in Figure 13 (Appendix, Table 2). There were no significant differences between the sham-operated and the intact control groups at either five or ten days post-urophysectomy. There was a significant decrease in urine flow rate in the five day urophysectomized fish compared to the sham-operated and intact controls for that time period. The urine flow of the ten day urophysectomized animals did not differ significantly from the values obtained for the sham and intact control groups. The urine

FIGURE 12. The effect of urophysectomy on plasma calcium concentration in goldfish at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

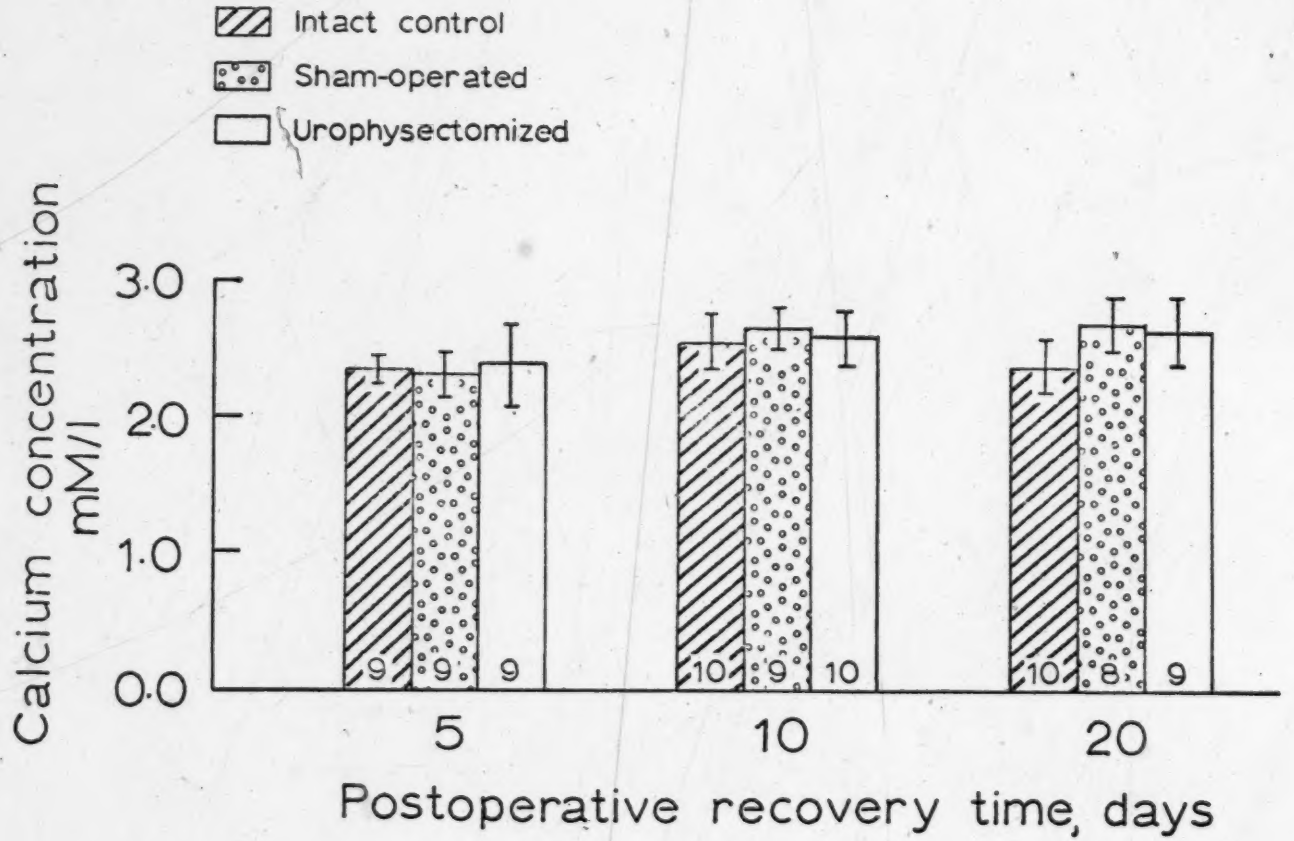
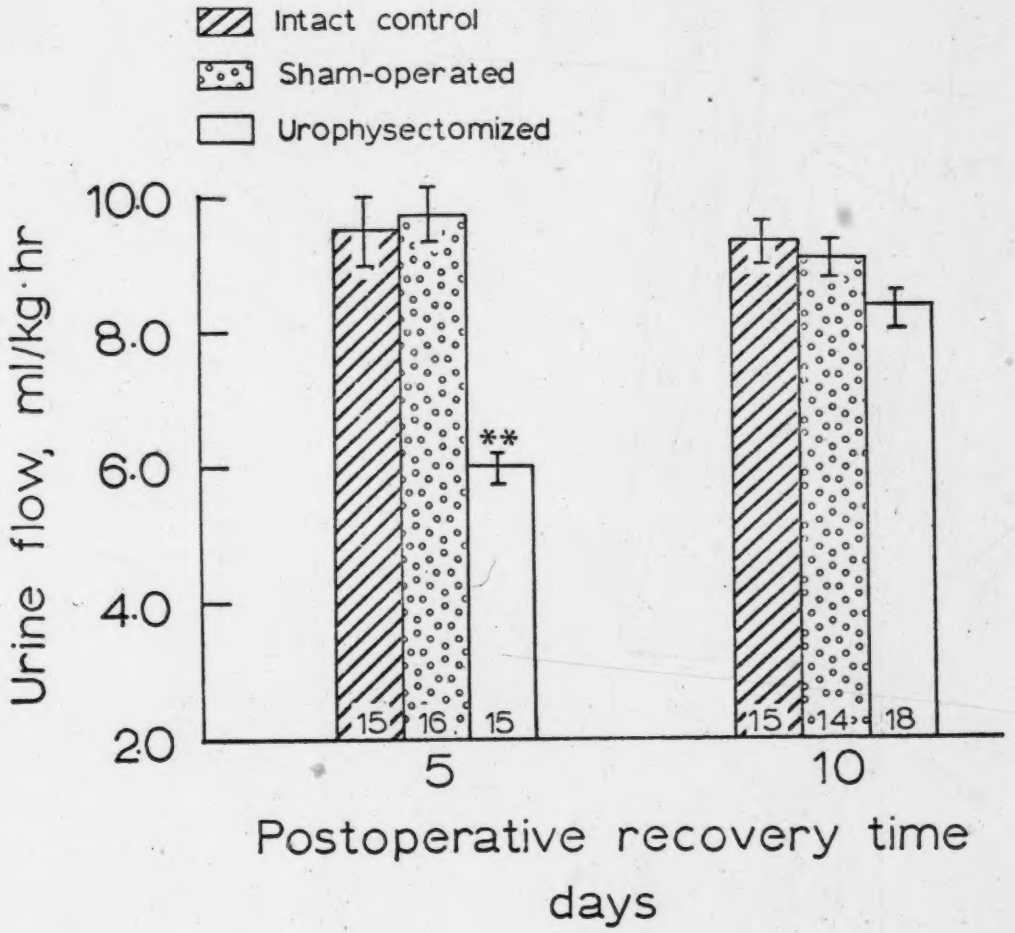

Intact control
Sham-operated
Urophysectomized
Calcium concentration mM/l
3.0
2.0
1.0
0.0
9
9
9
10
9
10
10
8
9
5
10
20
Postoperative recovery time, days

FIGURE 13. The effect of urophysectomy on the urine flow of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Urophysectomized
Urine flow, ml/kg·hr
10.0
8.0
6.0
4.0
2.0
**
15
16
15
15
14
18
5
10
Postoperative recovery time
days

flow of the five day urophysectomized fish was significantly lower ($p<0.01$) than for the ten day urophysectomized fish, indicating that the decrease was transient.

As shown in Figure 14 (Appendix, Table 2), urine osmolality was not changed significantly by urophysectomy. The sham-operated animals had an average urine osmolality corresponding to the intact controls at both the five and ten day postoperative periods. The average urine osmolality for the five day urophysectomized fish was slightly, but not significantly, lower than that of the sham and intact control groups. There was no difference in the urine osmolality of the ten day urophysectomized fish and the respective sham and intact controls, nor was there any significant difference between the five and ten day urophysectomized fish.

Urophysectomy did not affect the urine Na^+ concentration (U_{Na}) of the goldfish in this study (Figure 15) (Appendix, Table 2). The urine Na^+ concentration did not vary significantly between the sham-operated and intact control groups at either postoperative time periods. The urine Na^+ concentration of the five and ten day urophysectomized animals was not significantly lower than that of their respective sham-operated and intact control groups. The urine Na^+ levels of the five and ten day urophysectomized groups were also not significantly different.

FIGURE 14. The effect of urophysectomy on the osmolality (mOsm/l) of goldfish urine at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

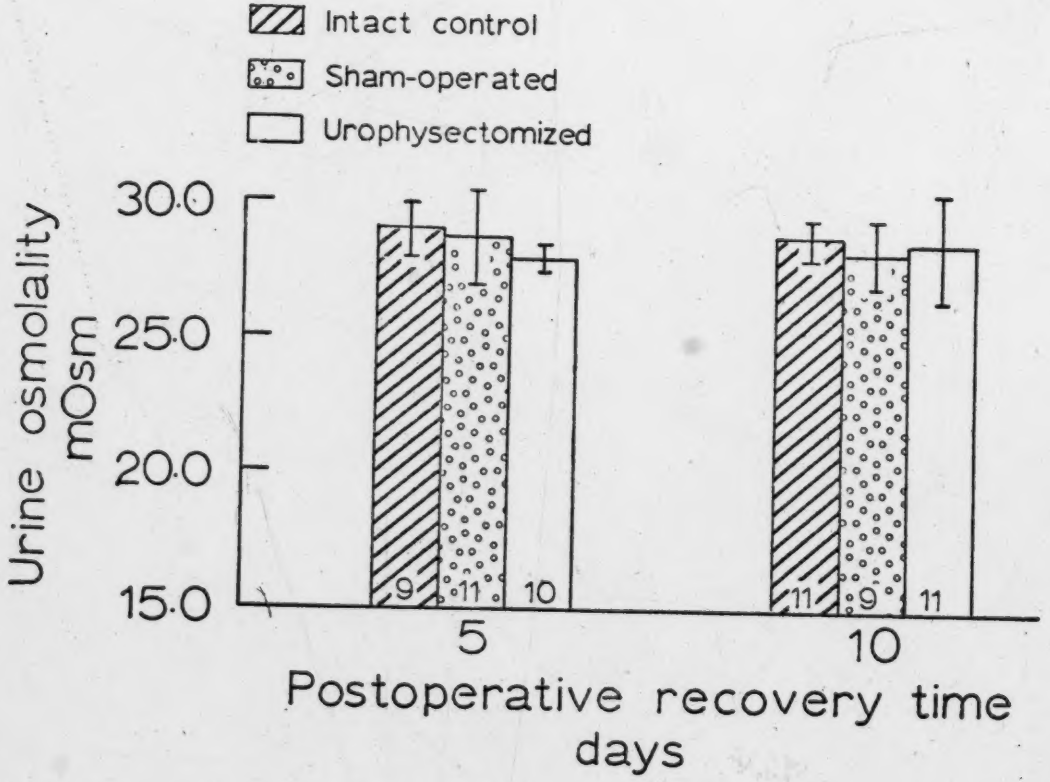
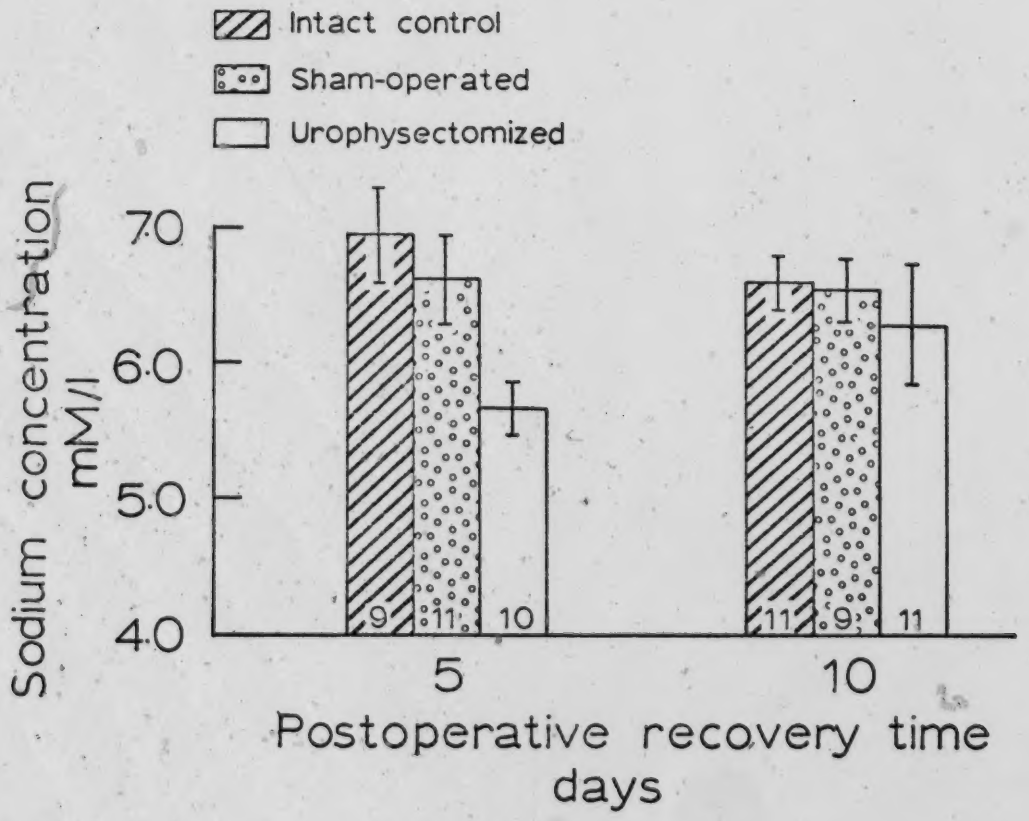

Intact control
Sham-operated
Urophysectomized
Urine osmolality
mOsm
30.0
25.0
20.0
15.0
9
11
10
11
9
11
5
10
Postoperative recovery time
days

FIGURE 15. The effect of urophysectomy on the urine sodium concentration of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.


Intact control
Sham-operated
Urophysectomized
Sodium concentration mM/l
7.0
6.0
5.0
4.0
9
11
10
11
9
11
5
10
Postoperative recovery time
days

As a reflection of the decreased urine flow, Na^+ excretion rate ($V \cdot U_{Na}$) was also significantly lower in the urophysectomized fish five days post-urophysectomy when compared to the sham and intact control animals (Figure 16) (Appendix, Table 2). The sham-operated animals maintained Na^+ excretion rates similar to the intact control fish throughout this study. The average rate of Na^+ excretion in the five day urophysectomized fish was reduced to 31.5 ± 1.3 μm/kg·hr, which was approximately fifty percent of the Na^+ excreted by the sham or intact controls. There was no significant difference in Na^+ excretion rate between the ten day urophysectomized, sham-operated or intact control groups. The rate of Na^+ excretion was significantly lower in the five day urophysectomized fish ($p<0.001$) compared to the ten day urophysectomized animals.

Urophysectomy had no significant effects on urine Cl^- levels (U_{Cl}) (Figure 17) (Appendix, Table 2). The sham-operated group had urine Cl^- levels which were not significantly different from the intact control groups. The urine Cl^- concentration of the five day urophysectomized fish, though somewhat lower, was not significantly different than that of the five day sham and intact control fish and the ten day urophysectomized animals. The urine Cl^- levels of the ten day urophysectomized fish were also not significantly different from their respective sham-operated or

FIGURE 16. The effect of urophysectomy on sodium excretion rates of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

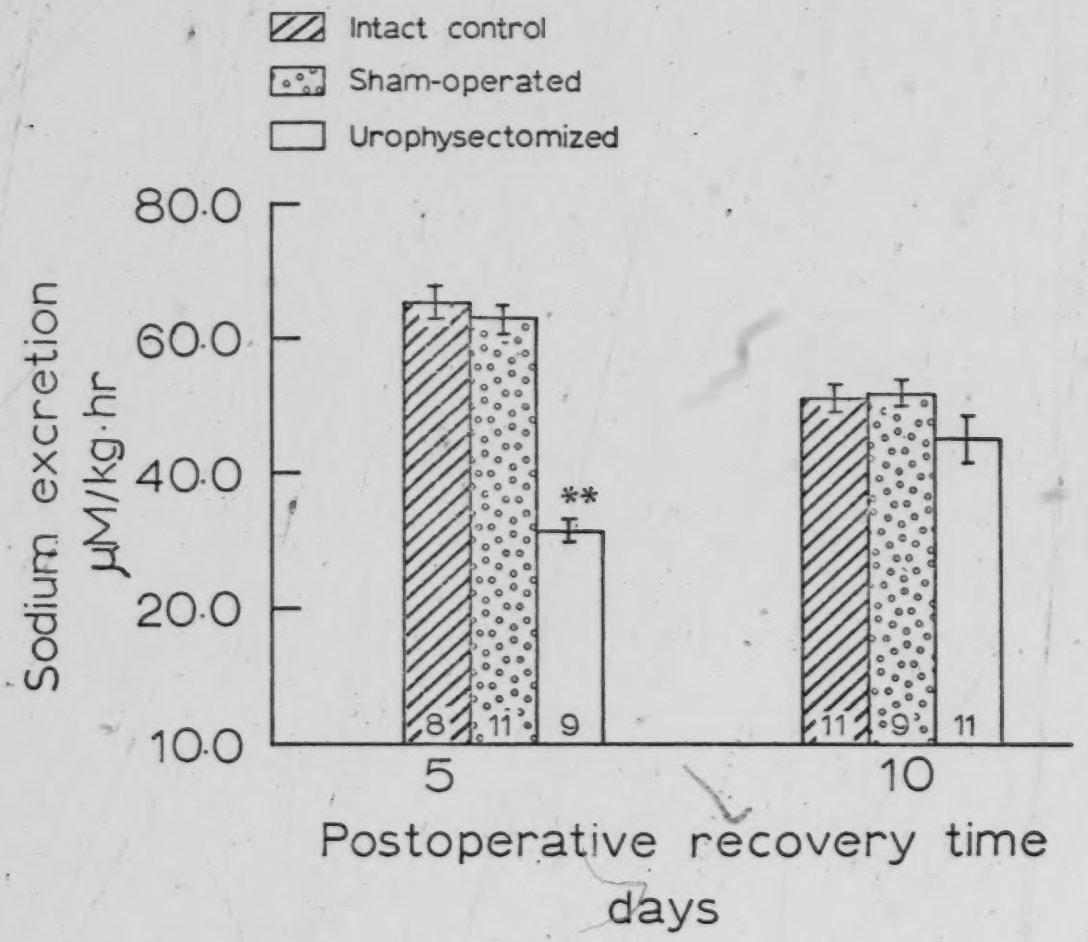
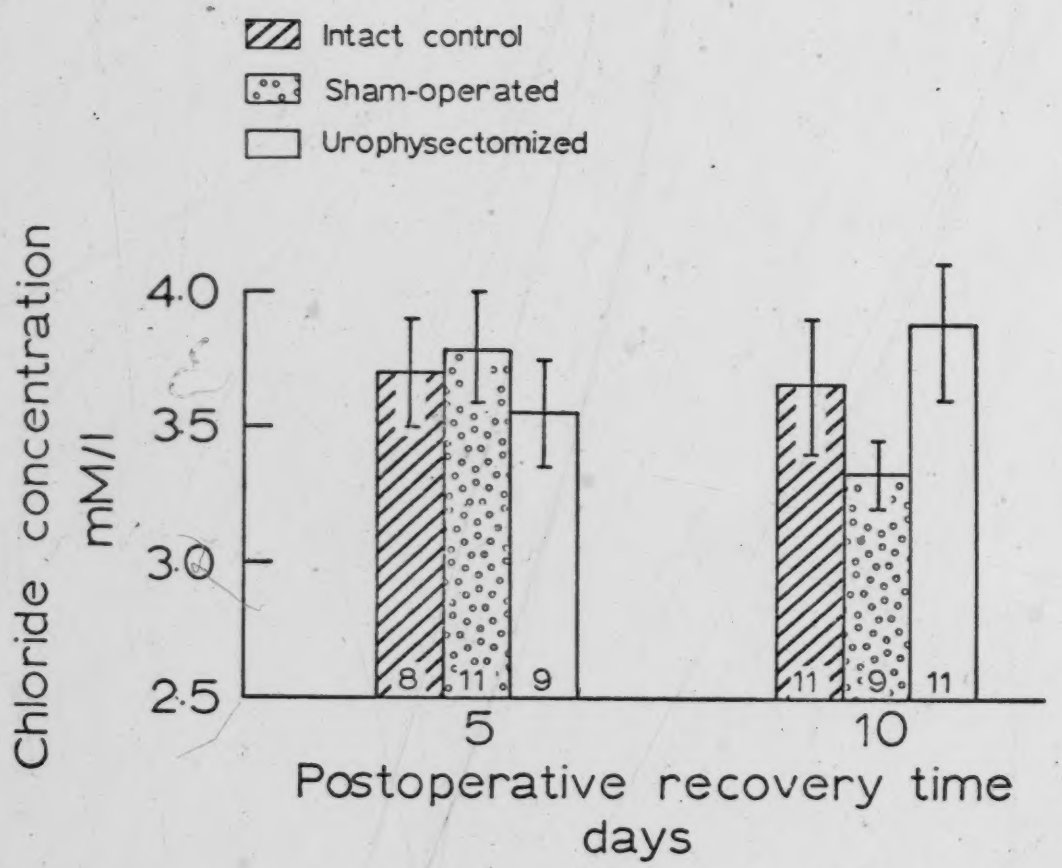

Intact control
Sham-operated
Urophysectomized
Sodium excretion
μM/kg·hr
80.0
60.0
40.0
20.0
10.0
**
8
11
9
11
9
11
5
10
Postoperative recovery time
days

FIGURE 17. The effect of urophysectomy on urine chloride concentrations of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.


Intact control
Sham-operated
Urophysectomized
Chloride concentration
mM/l
4·0
3·5
3·0
2·5
8
11
9
11
9
11
5
10
Postoperative recovery time
days

intact control groups.

Urophysectomy resulted in a significant decrease in the total amount of Cl^- excreted ($V \cdot U_{Cl}$) in the five day urophysectomized fish compared to their sham and intact control groups (Figure 18) (Appendix, Table 2). This reflects the decrease in urine flow rate for the urophysectomized fish. There was no significant difference in the Cl^- excretion rate between the sham-operated animals and the intact controls throughout this study. The ten day urophysectomized fish had a Cl^- excretion rate similar to that of the sham and intact controls. Cl^- excretion was significantly lower in the five day urophysectomized fish ($p<0.05$) compared to the ten day urophysectomized fish, again reflecting the decreased urine flow in the five day operated fish.

The urine Ca^{++} concentration (U_{Ca}) and Ca^{++} excretion ($V \cdot U_{Ca}$) rate of the five and ten day sham and intact control groups were not significantly different (Figures 19 and 20) (Appendix, Table 2). There was a significant decrease in both urine Ca^{++} concentration and the rate of Ca^{++} excretion in the five day urophysectomized fish compared to the sham and intact control groups for that postoperative time period. There was, however, no difference in the urine Ca^{++} concentration or Ca^{++} excretion rate of the ten day urophysectomized fish and their

FIGURE 18. The effect of urophysectomy on the urine chloride excretion rates of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

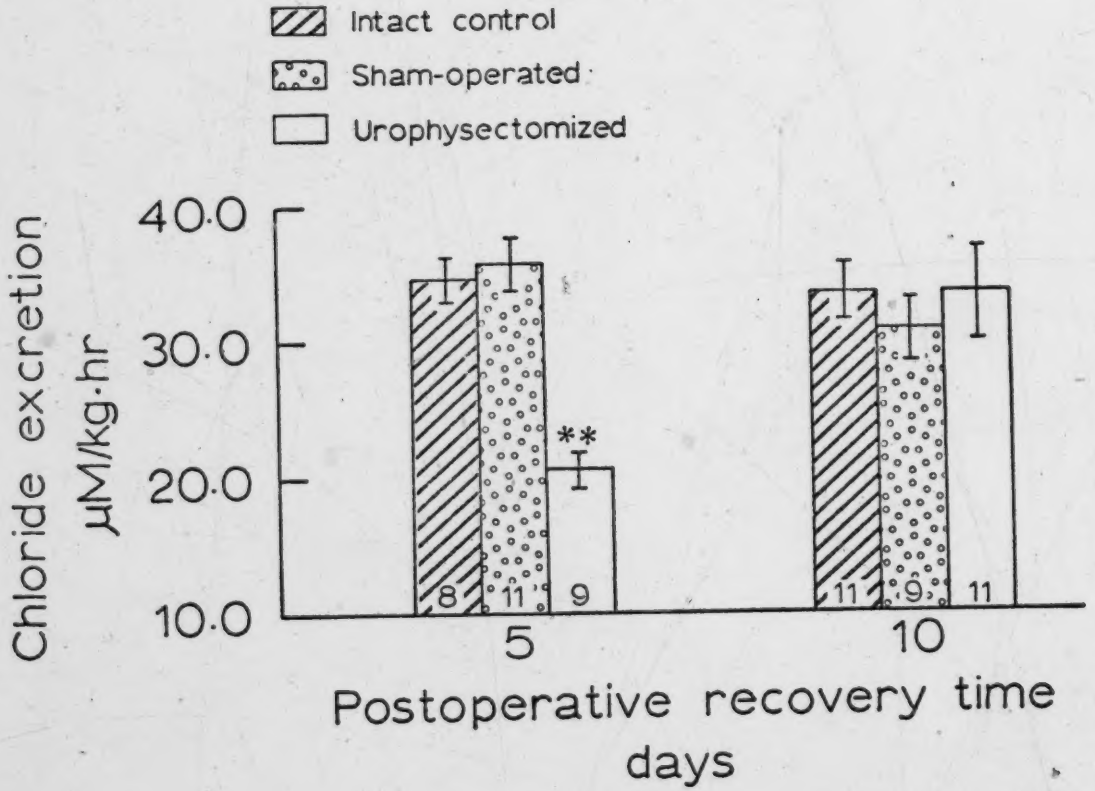

Intact control
Sham-operated
Urophysectomized
Chloride excretion
μM/kg·hr
40.0
30.0
20.0
10.0
**
8
11
9
11
9
11
5
10
Postoperative recovery time
days

FIGURE 19. The effects of urophysectomy on the urine calcium concentration of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is given at the base of each bar.

* $p < 0.05$, comparing the means of operated and control groups.

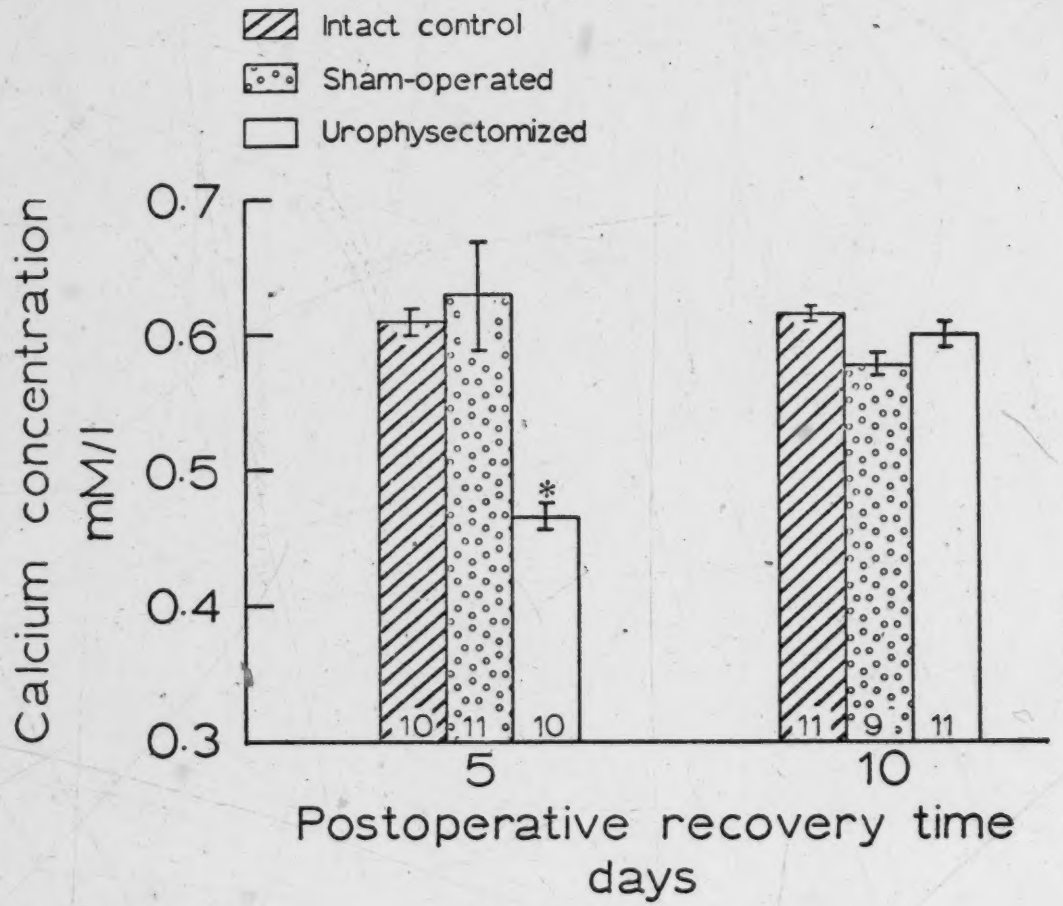
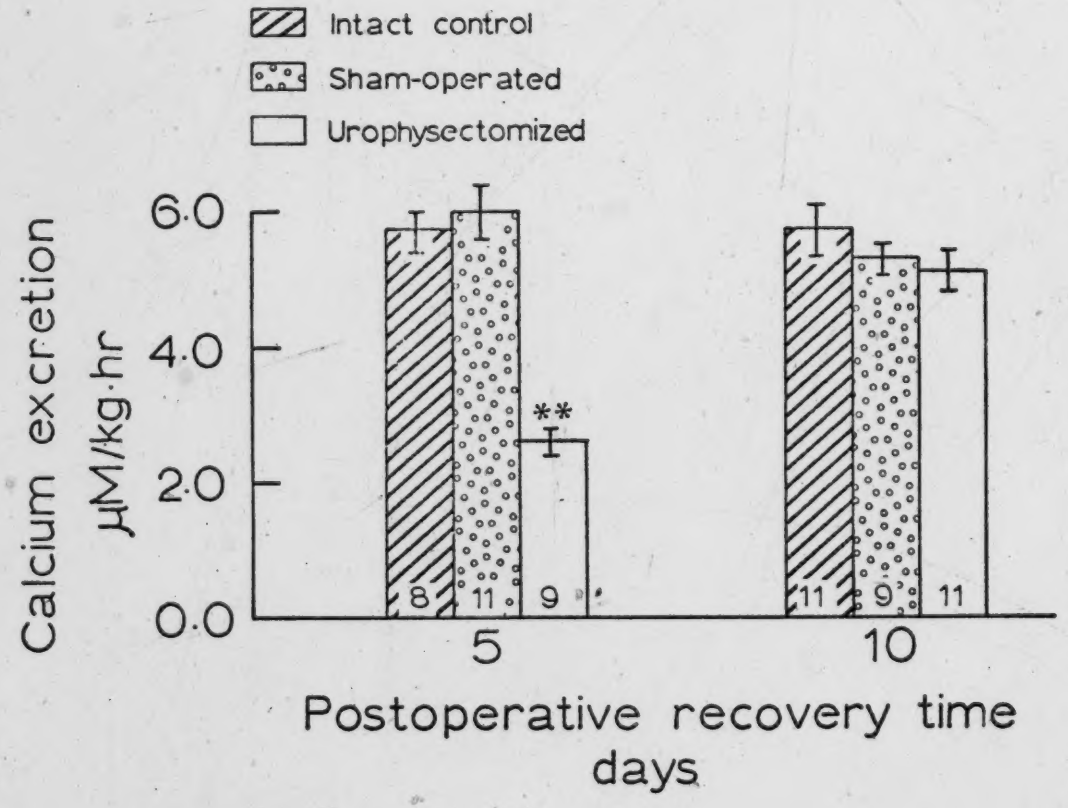

Intact control
Sham-operated
Urophysectomized
Calcium concentration
mM/l
0·7
0·6
0·5
0·4
0·3
10
11
10
11
9
11
*
5
10
Postoperative recovery time
days

FIGURE 20. The effect of urophysectomy on the calcium excretion rate of the goldfish at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Urophysectomized
Calcium excretion
μM/kg·hr
6.0
4.0
2.0
0.0
**
8
11
9
11
9
11
5
10
Postoperative recovery time
days

respective sham and intact control groups. The urine Ca^{++} level and the rate of Ca^{++} excretion of the five day urophysectomized fish were significantly lower than the ten day urophysectomized fish ($p<0.01$ and $p<0.01$ respectively).

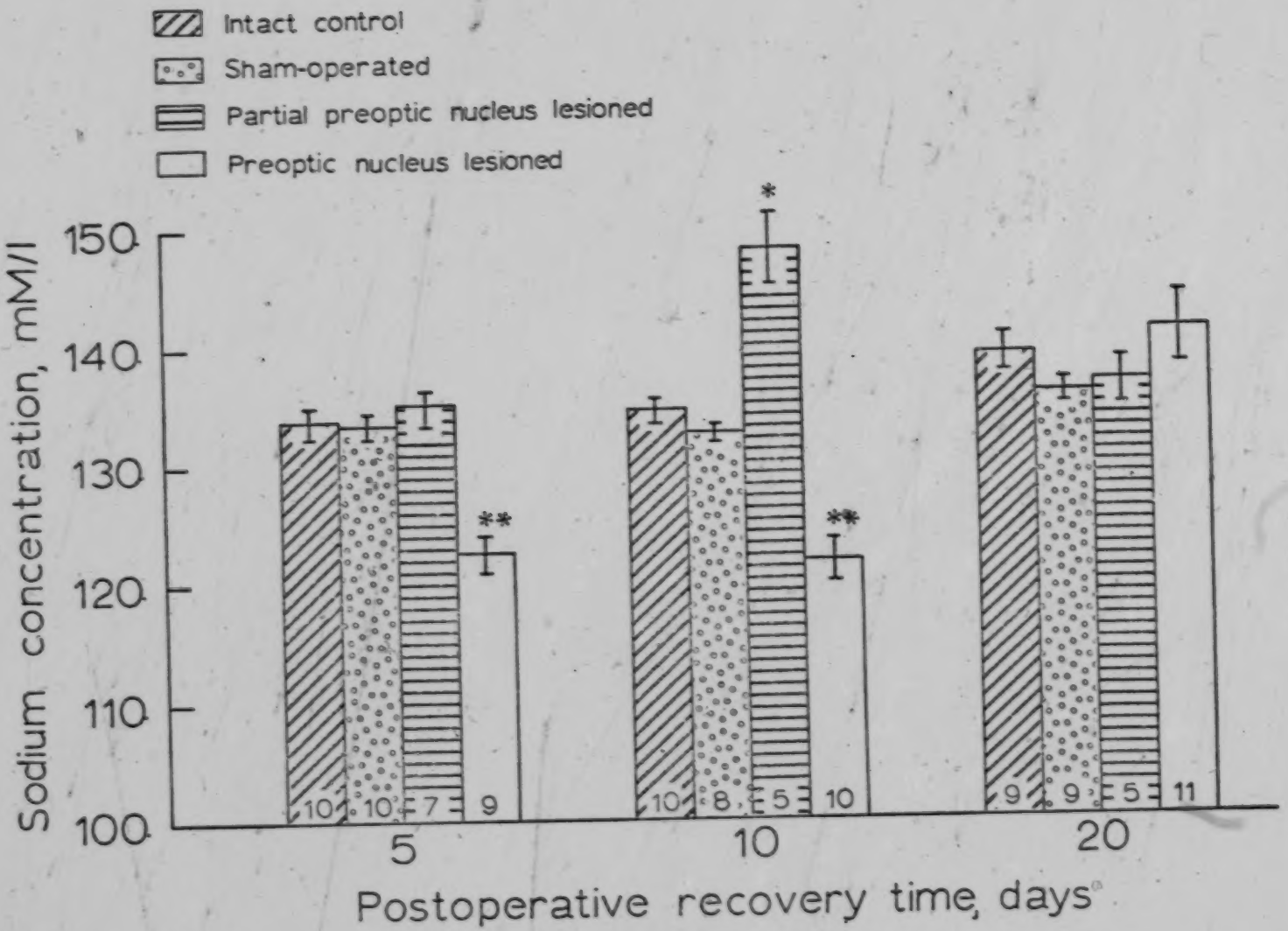
Preoptic Nucleus Lesion

Plasma Electrolyte Levels

The effects of lesioning the NPO on the plasma Na^{+} concentration are shown in Figure 21 (Appendix, Table 3). The sham-operated and intact control fish maintained similar plasma Na^{+} levels at all postoperative sampling times. The plasma Na^{+} levels in the five and ten day NPO lesioned fish (complete lesion) were significantly lower than in the sham and intact controls at the same postoperative time periods. The twenty day completely lesioned animals had plasma Na^{+} concentrations similar to the sham and intact control values. A significant difference in the plasma Na^{+} concentration was found between the completely and those partially lesioned at both five and ten days postoperatively ($p<0.01$) but not at the twenty day sampling period. The five and twenty day partially lesioned fish had plasma Na^{+} levels similar to their respective sham and intact control groups. However, the ten day partially lesioned group had a plasma Na^{+} level significantly higher ($p<0.05$) than for the ten day sham-operated and intact controls. The plasma Na^{+} levels of the five and

FIGURE 21. The effect of lesioning the preoptic nucleus of the goldfish on plasma sodium concentration at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

* $p < 0.05$, ** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Partial preoptic nucleus lesioned
Preoptic nucleus lesioned
Sodium concentration, mM/l
150
140
130
120
110
100
*
**
**
10
10
7
9
10
8
5
10
9
9
5
11
5
10
20
Postoperative recovery time, days

ten day completely lesioned fish were not significantly different. Both the five and ten day completely lesioned fish were, however, significantly lower than the twenty day completely lesioned fish ($p<0.01$ and $p<0.01$ respectively).

Plasma Cl^- levels were quite variable (Figure 22) (Appendix, Table 3). There was a large range in plasma Cl^- concentrations among the individuals within an experimental group. No significant differences were detected between the sham-operated and intact controls at any of the postoperative recovery times. There were no significant differences in plasma Cl^- concentration between the five, ten and twenty day completely lesioned groups and their corresponding sham and intact controls. There also was no significant differences in plasma Cl^- levels between the five, ten and twenty day completely lesioned groups, although there was some decrease in the Cl^- level of the twenty day lesioned animals. The partially lesioned animals showed no differences in plasma Cl^- concentrations from one postoperative sampling time to another and were not significantly different from the completely lesioned animals for the same postoperative time periods.

NPO lesioning did not significantly alter plasma Ca^{++} concentrations (Figure 23) (Appendix, Table 3). The sham-operated and the intact control animals were not

FIGURE 22. The effect of lesioning the preoptic nucleus of goldfish on the plasma chloride concentrations at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

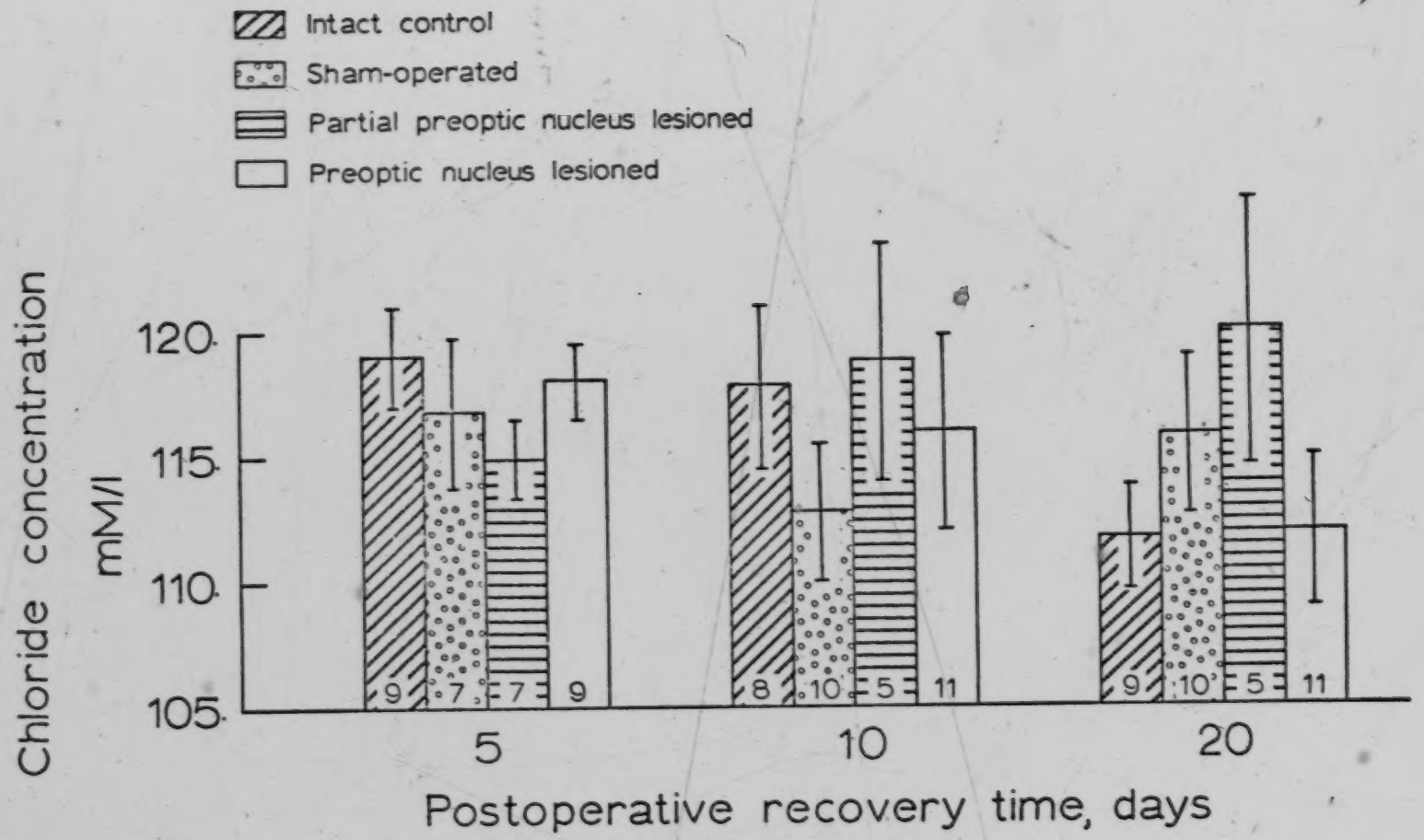
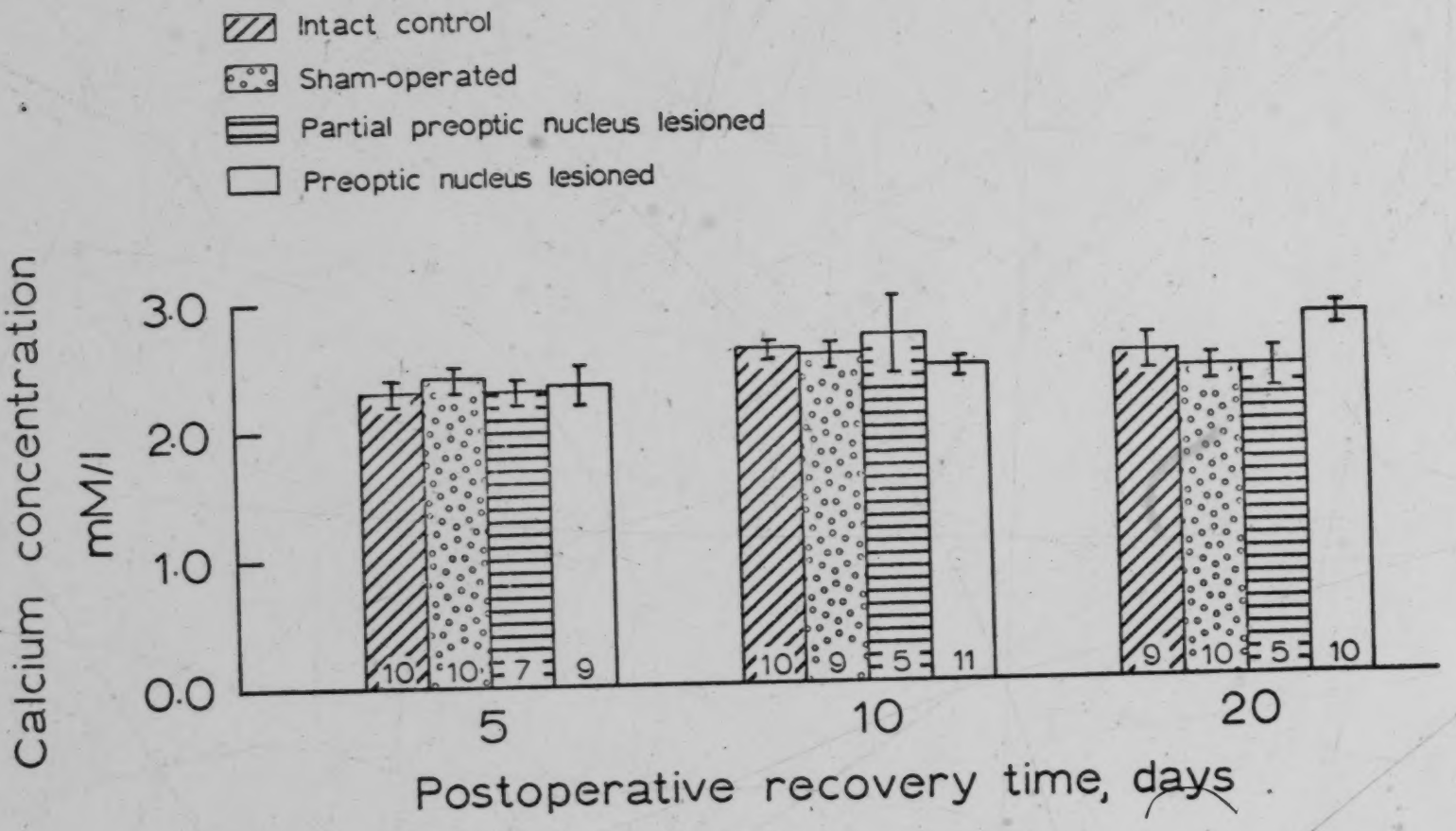

Intact control
Sham-operated
Partial preoptic nucleus lesioned
Preoptic nucleus lesioned
Chloride concentration
mM/l
120
115
110
105
9
7
7
9
8
10
5
11
9
10
5
11
5
10
20
Postoperative recovery time, days

FIGURE 23. The effect of lesioning the preoptic nucleus of the goldfish on plasma calcium concentrations at five, ten and twenty days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.


Intact control
Sham-operated
Partial preoptic nucleus lesioned
Preoptic nucleus lesioned
Calcium concentration
mM/l
3.0
2.0
1.0
0.0
10
10
7
9
10
9
5
11
9
10
5
10
5
10
20
Postoperative recovery time, days

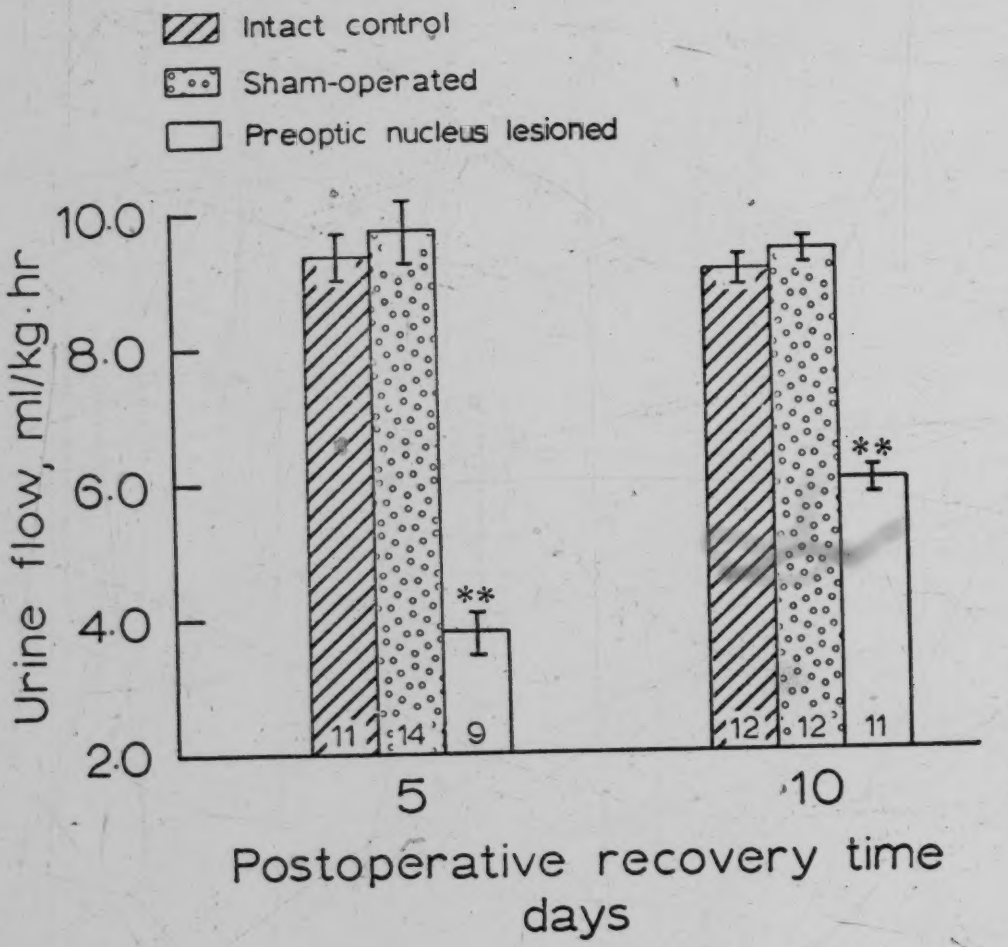
significantly different at any of the postoperative sampling times. Plasma Ca^{++} levels of the five, ten and twenty day completely lesioned animals were not significantly different from their respective sham and intact control group values, nor were they significantly different from each other. There also were no significant differences between the partially lesioned animals at the five, ten and twenty day postoperative recovery periods and their respective sham and intact controls.

Renal Study

Lesioning of the NPO produced a dramatic antidiuretic effect on both the five and ten day lesioned fish (Figure 24) (Appendix, Table 4). The sham-operated groups had urine flow rates (V) that were not significantly different than the intact controls at either postoperative time period. The average urine flow in the five day lesioned fish was reduced to approximately forty percent of that of the sham-operated and intact controls. The ten day lesioned fish had an average urine flow approximately fifty percent of that of the sham and the intact control groups. Urine flow was significantly lower ($p<0.05$) in the five day lesioned fish compared to the ten day lesioned fish. However, urine flow in the ten day lesioned animals was comparable to that found for the five day urophysectomized fish (see above). There were no incompletely

FIGURE 24. The effect of lesioning the preoptic nucleus of goldfish on urine flow at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Preoptic nucleus lesioned
Urine flow, ml/kg·hr
10·0
8·0
6·0
4·0
2·0
**
**
11
14
9
12
12
11
5
10
Postoperative recovery time
days

lesioned animals in the renal study.

Urine osmolality significantly increased as a result of lesioning the NPO (Figure 25) (Appendix, Table 4). There were no differences in urine osmolality between the sham-operated fish and the intact controls at either five or ten days postoperatively. Urine osmolality was, however, significantly higher in both the five and ten day lesioned groups compared to the respective sham and intact control groups. No significant differences in urine osmolality between the five and ten day lesioned groups were found.

There was a significant increase in urine Na^+ concentration (U_{Na}) as a result of lesioning the NPO (Figure 26) (Appendix, Table 4). There was no difference in urine Na^+ concentration between the sham-operated and the intact controls at either the five or ten day postoperative sampling times. Urine Na^+ concentration was significantly increased in the lesioned fish at both five and ten days postoperatively compared to the respective sham and control groups. There was, however, no difference between the lesioned groups.

No significant differences in the Na^+ excretion rates ($V \cdot U_{Na}$) were found between the sham-operated and the intact control groups in this experiment (Figure 27) (Appendix, Table 4). While the rate of Na^+ excretion in the

FIGURE 25. The effect of lesioning the preoptic nucleus of the goldfish on urine osmolality at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

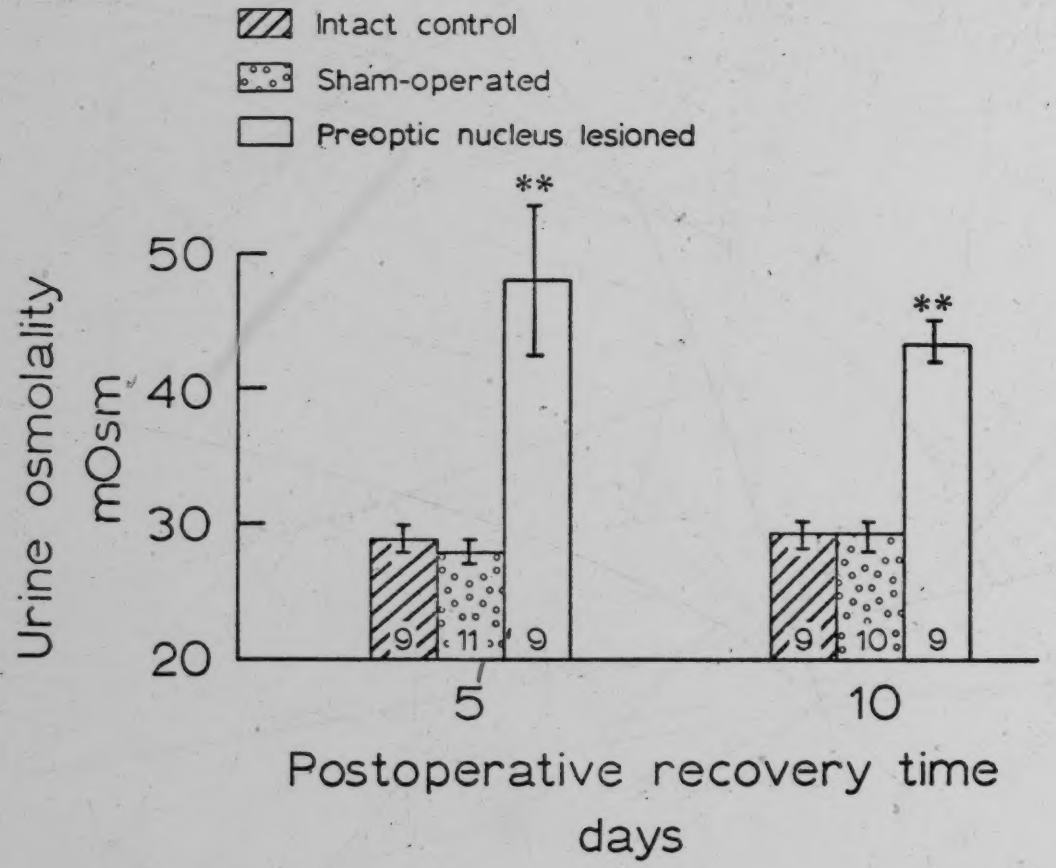

Intact control
Sham-operated
Preoptic nucleus lesioned
Urine osmolality
mOsm
50
40
30
20
**
**
9
11
9
9
10
9
5
10
Postoperative recovery time
days

FIGURE 26. The effect of lesioning the preoptic nucleus of the goldfish on urine sodium concentration at five and ten days post-operatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is given at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

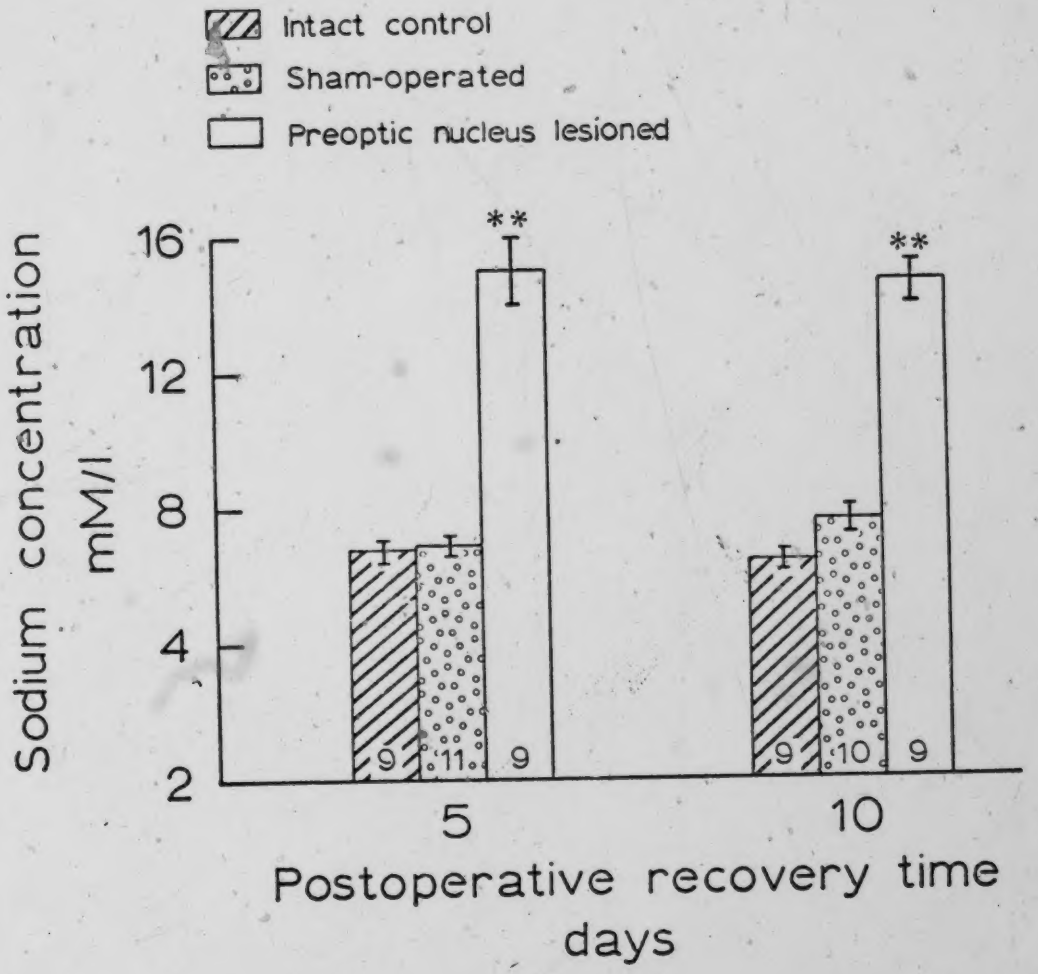
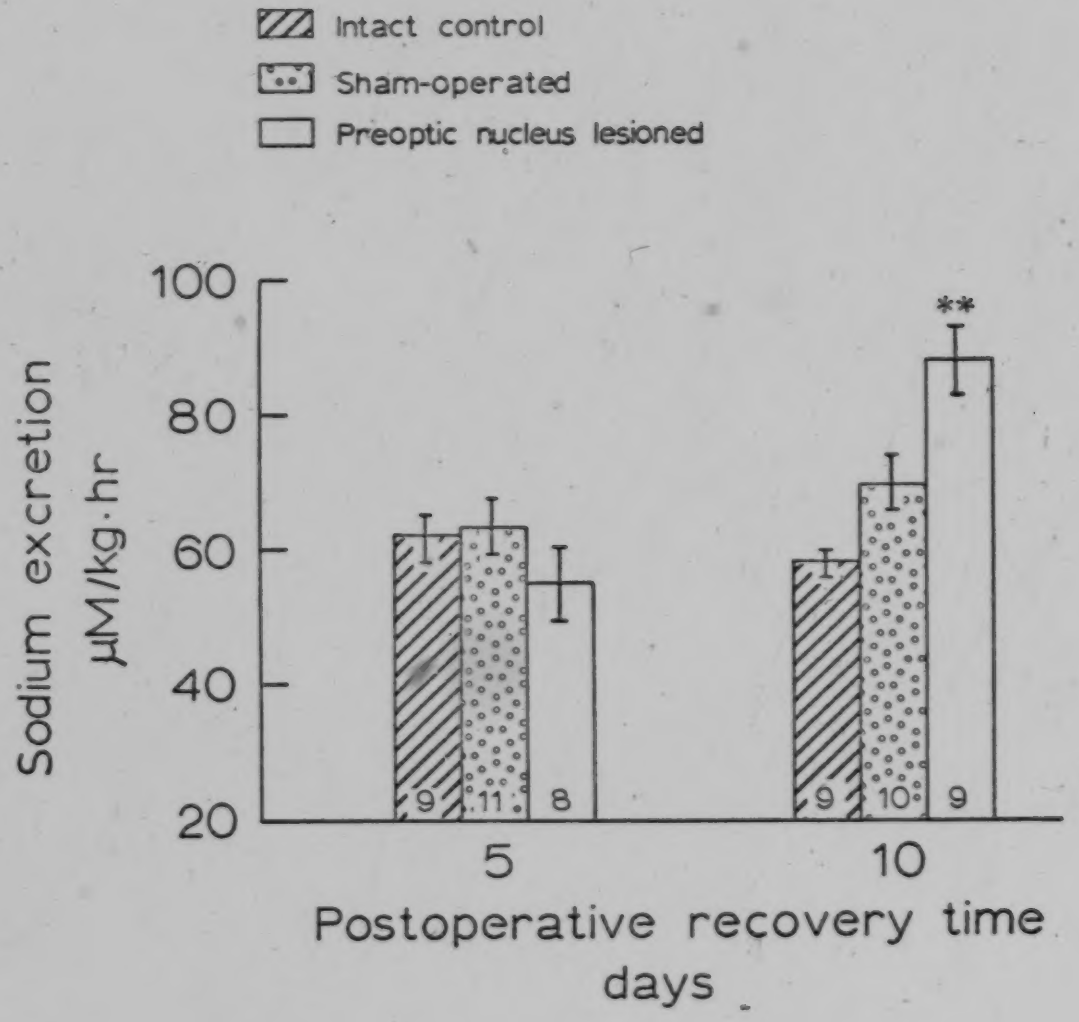

Intact control
Sham-operated
Preoptic nucleus lesioned
Sodium concentration
mM/l
16
12
8
4
2
**
**
9
11
9
9
10
9
5
10
Postoperative recovery time
days

FIGURE 27. The effect of lesioning the preoptic nucleus of the goldfish on urine sodium excretion rates, at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Preoptic nucleus lesioned
Sodium excretion
μM/kg·hr
100
80
60
40
20
**
9
11
8
9
10
9
5
10
Postoperative recovery time
days

ten day lesioned fish increased significantly compared to the ten day sham-operated and intact controls, the Na^+ excretion rate in the five day lesioned animals was not significantly different from the control groups. This is a result of the combined effect of a reduced urine flow and an increased urine Na^+ concentration in the five day lesioned fish. Na^+ excretion in the five day lesioned fish was significantly lower ($p<0.01$) than the ten day lesioned group. This was due to an increase in urine flow in the ten day lesioned group.

There were no differences in the urine Cl^- levels (U_{Cl}) between the sham and intact control groups (Figure 28) (Appendix, Table 4). The urine Cl^- concentration was significantly higher in the five day and ten day lesioned animals than in the respective sham-operated and intact control groups. No significant differences in the urine Cl^- levels between the two lesioned groups were observed.

The Cl^- excretion rates ($V \cdot U_{Cl}$) of the sham and intact controls were not significantly different at any postoperative sampling time (Figure 29) (Appendix, Table 4). There were also no significant differences in Cl^- excretion between the five day lesioned fish and the sham-operated and intact control groups. The ten day lesioned

FIGURE 28. The effect of lesioning the preoptic nucleus of the goldfish on urine chloride concentrations at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.

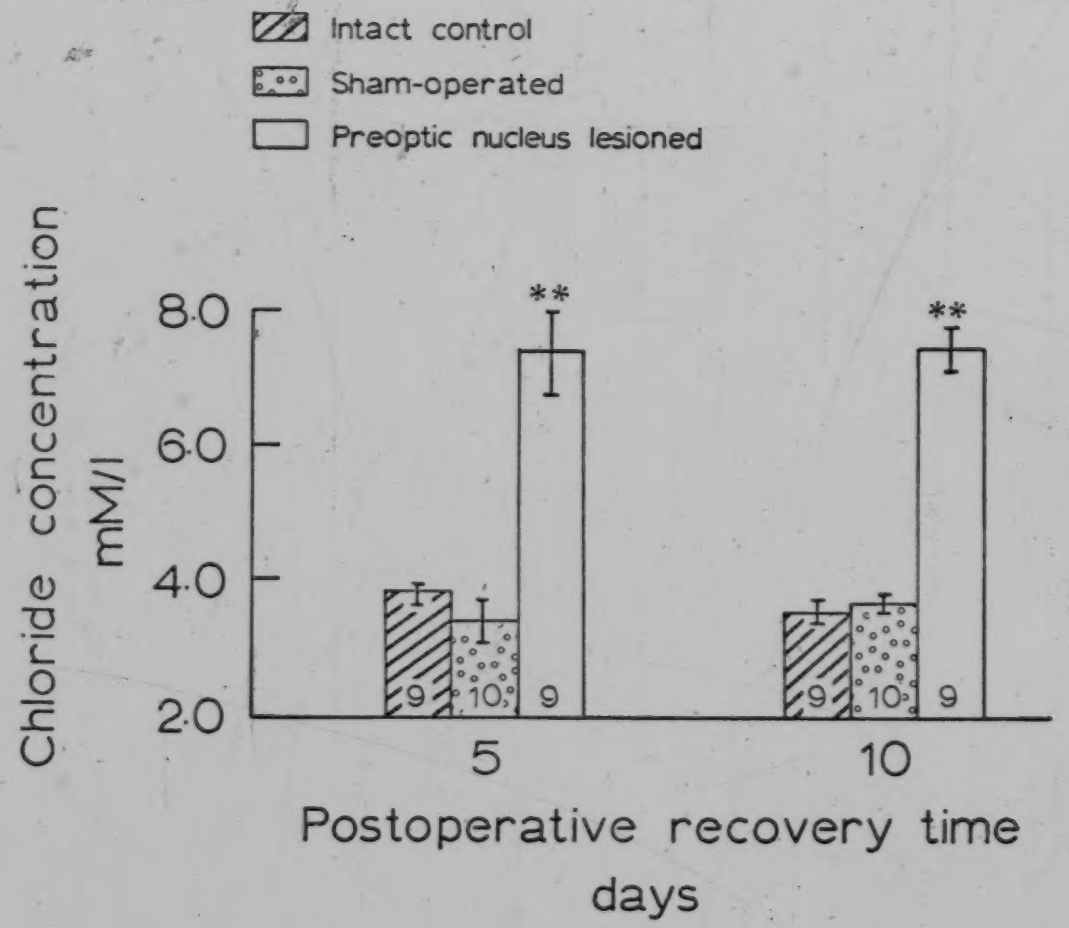
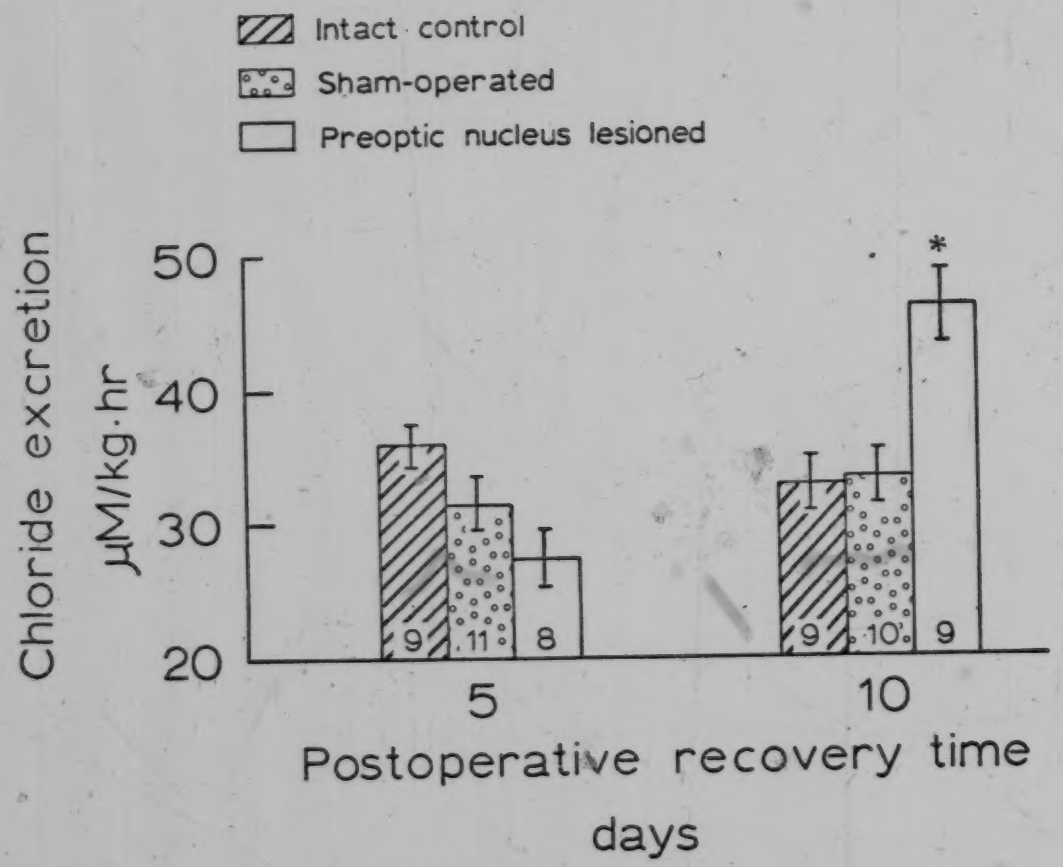

Intact control
Sham-operated
Preoptic nucleus lesioned
Chloride concentration
mM/l
8.0
6.0
4.0
2.0
**
**
9
10
9
9
10
9
5
10
Postoperative recovery time
days

FIGURE 29. The effect of lesioning the preoptic nucleus of the goldfish on urine chloride excretion rates at five and ten days postoperatively. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

* $p < 0.05$, comparing the means of operated and control groups.


Intact control
Sham-operated
Preoptic nucleus lesioned
Chloride excretion
μM/kg·hr
50
40
30
20
9
11
8
9
10
9
*
5
10
Postoperative recovery time
days

animals did, however, have a urine Cl^- excretion rate that was significantly higher than in either the sham-operated or the intact control fish. The Cl^- excretion rate was also significantly higher in the ten day lesioned animals ($p<0.01$) compared to the five day lesioned animals. Again, this is a reflection of the changes in urine flow.

The sham and intact control fish did not have urine Ca^{++} concentrations (U_{Ca}) that were significantly different at either postoperative recovery times (Figure 30) (Appendix, Table 4). Urine Ca^{++} levels were significantly higher in both the five and ten day lesioned fish compared to their respective sham and intact control groups. There was no significant difference between the lesioned groups.

There were no significant differences in Ca^{++} excretion ($V \cdot U_{Ca}$) between the sham-operated and the intact controls at either five or ten days postoperatively (Figure 31) (Appendix, Table 4). The five day lesioned fish had Ca^{++} excretion rates significantly lower than the sham-operated and intact control groups. Ca^{++} excretion was also significantly lower in the five day lesioned fish ($p<0.01$) than in the ten day lesioned animals. Again, this is a result of the reduction in urine flow in spite of an increase in Ca^{++} concentration of the urine.

FIGURE 30. The effect of preoptic nucleus lesioning of the goldfish on the urine calcium concentration at five and ten days postoperatively. The vertical bars represent ± SEM. The number of individuals in each experimental group is shown at the base of each bar.

* $p < 0.05$, comparing the means of operated and control groups.

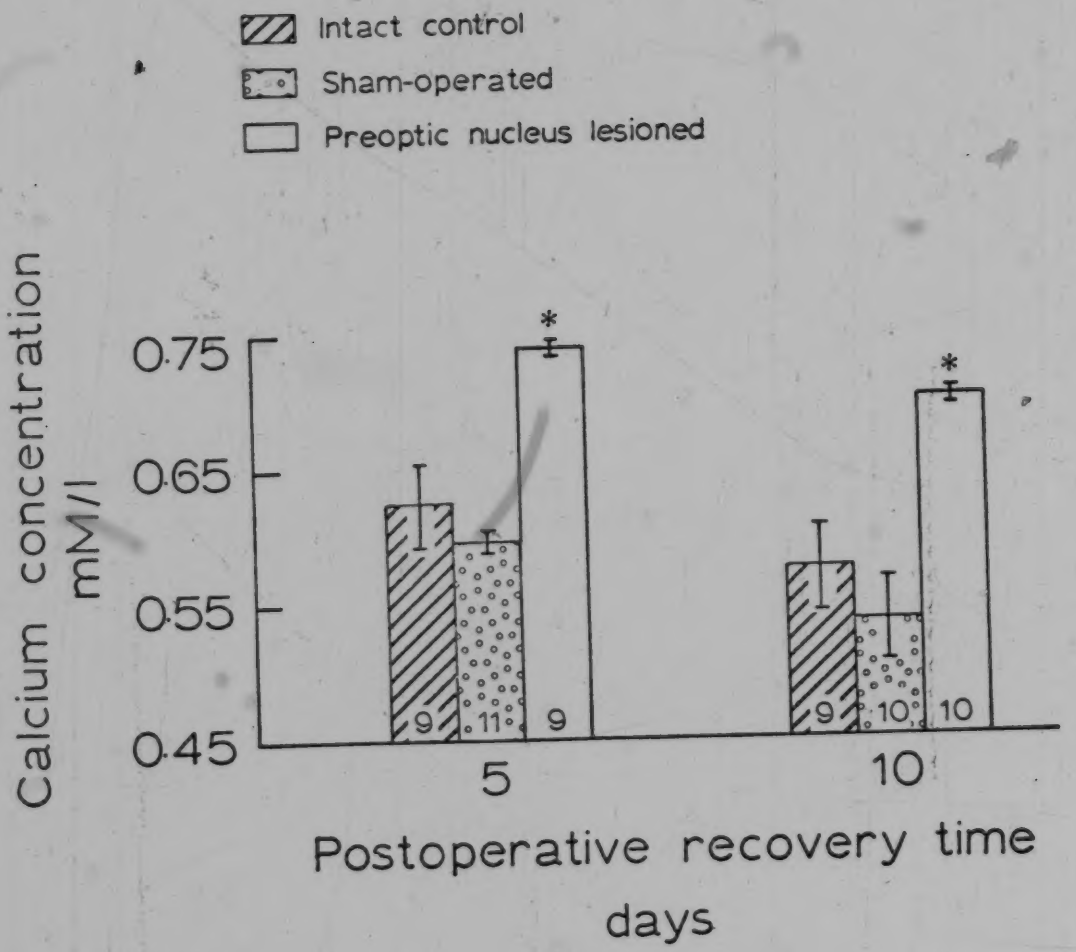
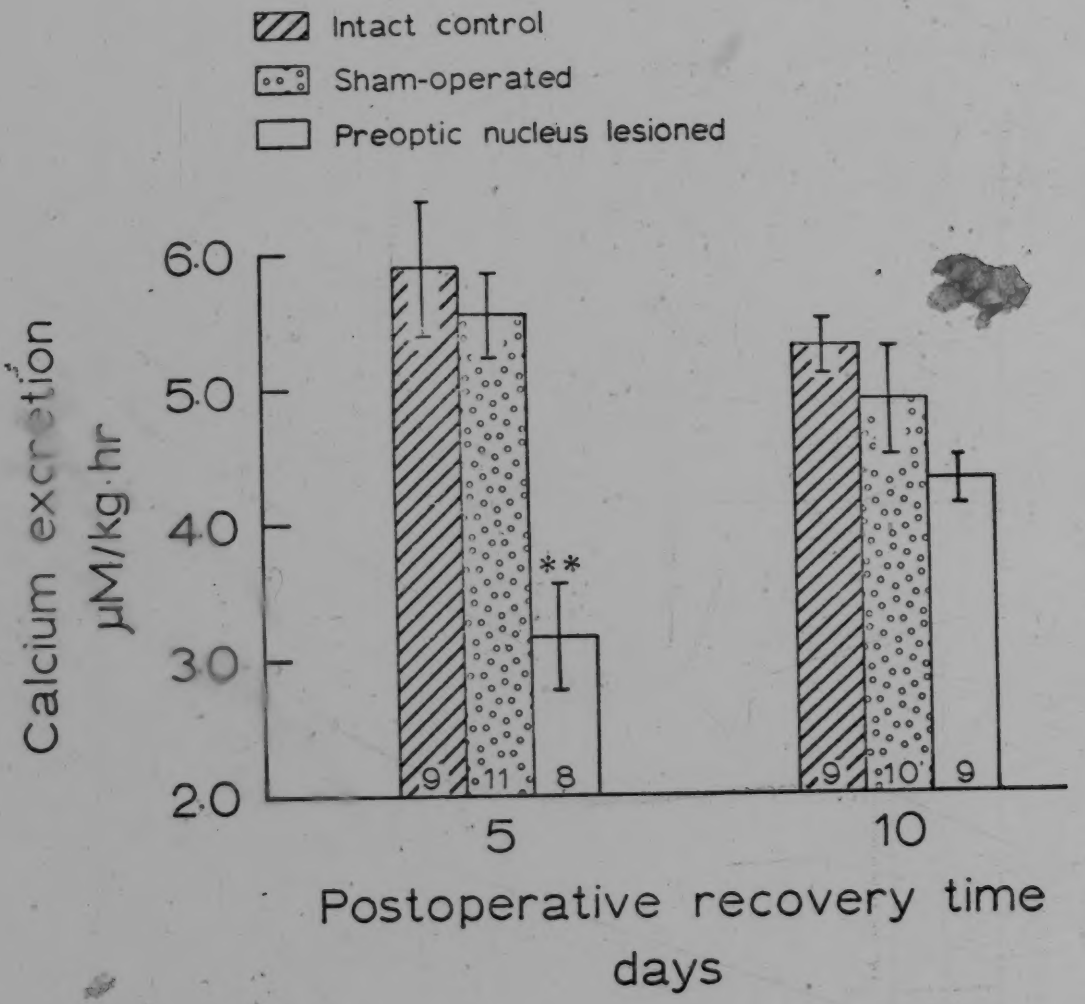

Intact control
Sham-operated
Preoptic nucleus lesioned
Calcium concentration mM/l
0·75
0·65
0·55
0·45
*
*
9
11
9
9
10
10
5
10
Postoperative recovery time
days

FIGURE 31. The effect of preoptic nucleus lesioning of the goldfish on urine calcium excretion rates at five and ten days post-operatively. The vertical bars represent ±SEM. The number of individuals in each experimental group (N) is shown at the base of each bar.

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Preoptic nucleus lesioned
Calcium excretion
µM/kg·hr
6·0
5·0
4·0
3·0
2·0
**
9
11
8
9
10
9
5
10
Postoperative recovery time
days

Preoptic Nucleus Lesion/Urophysectomy

Plasma Electrolyte Levels

The combined operations of NPO lesioning and urophysectomy caused a significant reduction in the plasma Na^+ levels of the five day completely lesioned/urophysectomized fish and partially lesioned/urophysectomized fish compared to the sham-operated and intact control groups (Figure 32) (Appendix, Table 5). The five day completely lesioned/urophysectomized animals and partially lesioned/urophysectomized animals showed no differences in plasma Na^+ concentration. The extremely low plasma Na^+ levels in the ten day completely lesioned/urophysectomized and the lowered levels in the sham and intact controls can be correlated with an accidental failure of the aquatic facilities three days prior to the ten day sampling time. High mortality of both experimental and control fish occurred as a result of the break-down. (The trauma and mortality was attributed to supersaturation of the water with oxygen and nitrogen and a sudden temperature increase.) Plasma Cl^- levels were also reduced in these same experimental groups (Figure 33). Results from the ten day postoperative sampling period should, therefore, be disregarded. By the twentieth postoperative day the plasma Na^+ concentration of the completely lesioned/urophysectomized fish was similar to the sham and intact control levels. There were no

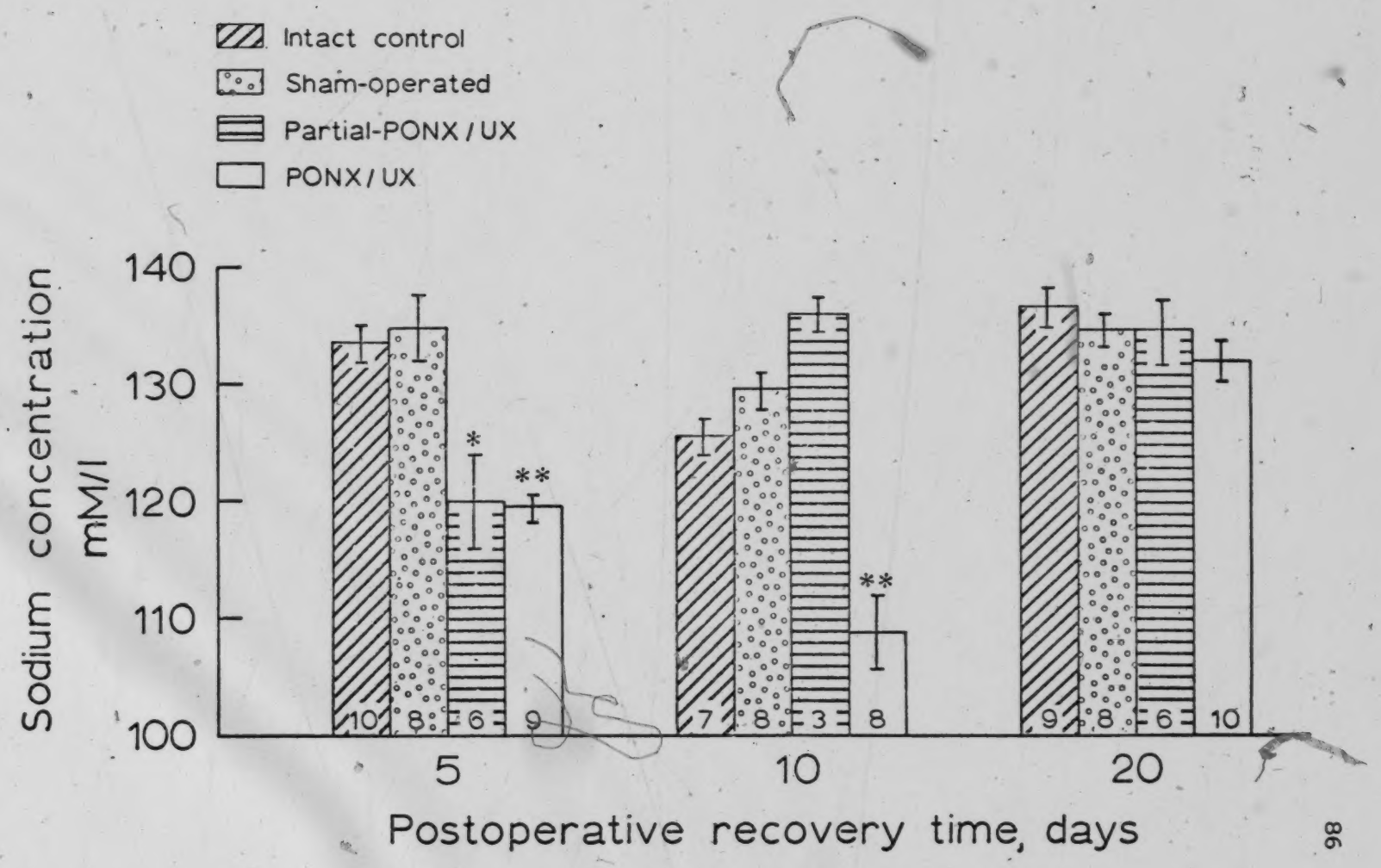
FIGURE 32. The effect of the combined operation of preoptic nucleus lesioning and urophysectomy on the plasma sodium concentrations in goldfish. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

Partial - PON/UX - partially preoptic nucleus lesioned and urophysectomized

PONX/UX - completely preoptic nucleus lesioned and urophysectomized

* $p < 0.05$

** $p < 0.01$, comparing the means of operated and control groups.


Intact control
Sham-operated
Partial-PONX / UX
PONX / UX
Sodium concentration
mM/l
140
130
120
110
100
*
**
**
10
8
6
9
7
8
3
8
9
8
6
10
5
10
20
Postoperative recovery time, days

significant differences in plasma Na^+ levels between the twenty day completely lesioned/urophysectomized and partially lesioned/urophysectomized fish. With the exception of the ten day groups the sham-operated and the intact control groups were not significantly different.

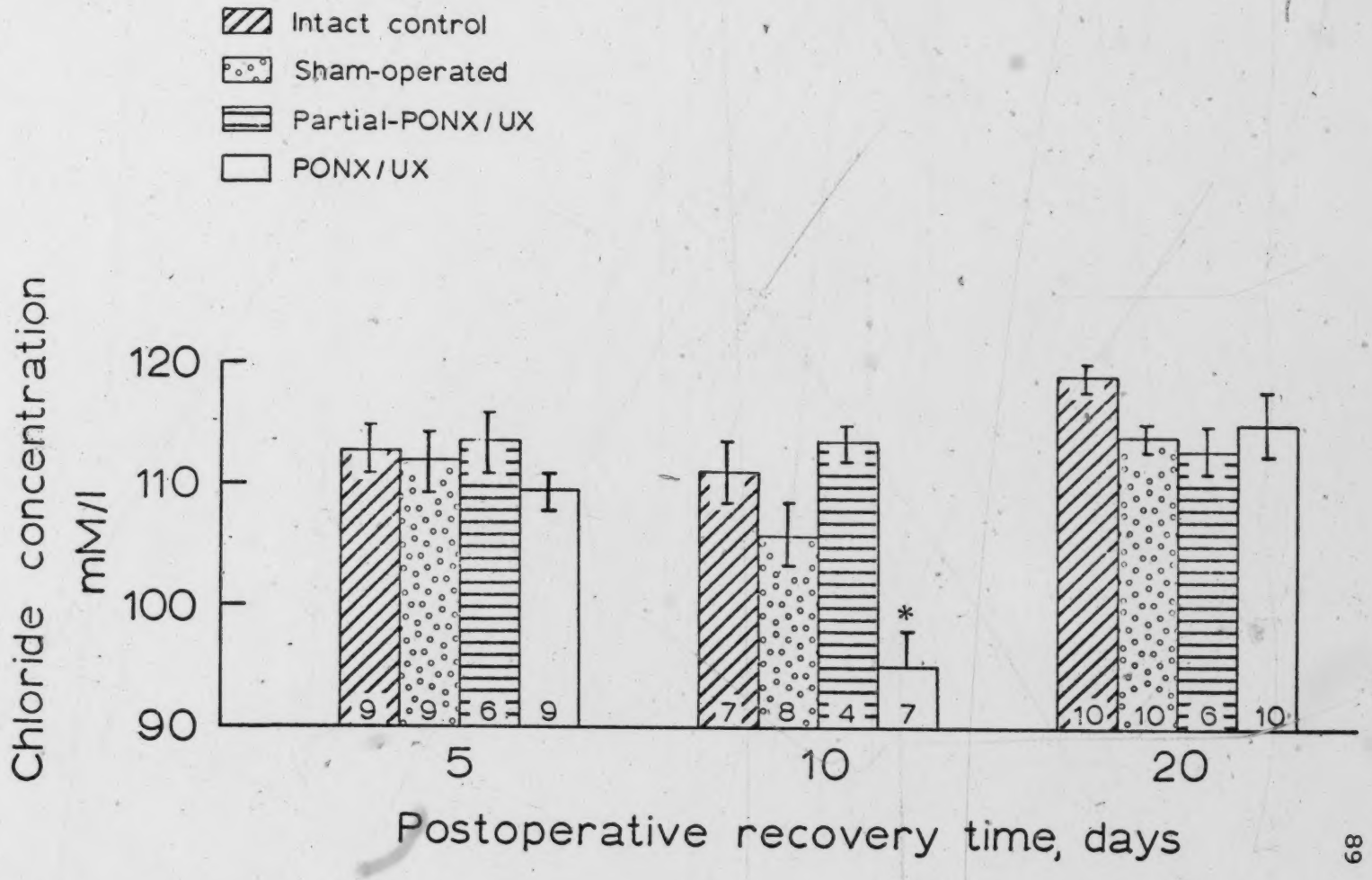
Plasma Cl^- levels were not changed by the simultaneous removal of the NPO and the urophysis (Figure 33) (Appendix, Table 5). The plasma Cl^- concentration for the five day completely lesioned/urophysectomized group was not significantly different from the five day sham and intact controls. There also were no significant differences between the five day completely lesioned/urophysectomized and the partially lesioned/urophysectomized animals. As previously stated, the Cl^- levels of all the ten day experimental groups should be disregarded. There were no significant differences between the twenty day completely lesioned/urophysectomized and the sham-operated control, the intact control or the partially lesioned/urophysectomized fish. Plasma Cl^- levels were also not significantly lower in the five day completely lesioned/urophysectomized fish compared to the twenty day completely lesioned/urophysectomized animals. There were no differences in plasma Cl^- concentration between the partially lesioned/urophysectomized fish at any of the postoperative recovery times.

FIGURE 33. The effect of the combined operation of preoptic nucleus lesioning and urophysectomy on the plasma chloride concentration in goldfish. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

Partial - PONX/UX - partially preoptic nucleus lesioned and urophysectomized

PONX/UX - completely preoptic nucleus lesioned and urophysectomized

* $p < 0.05$, comparing the means of operated and control groups.

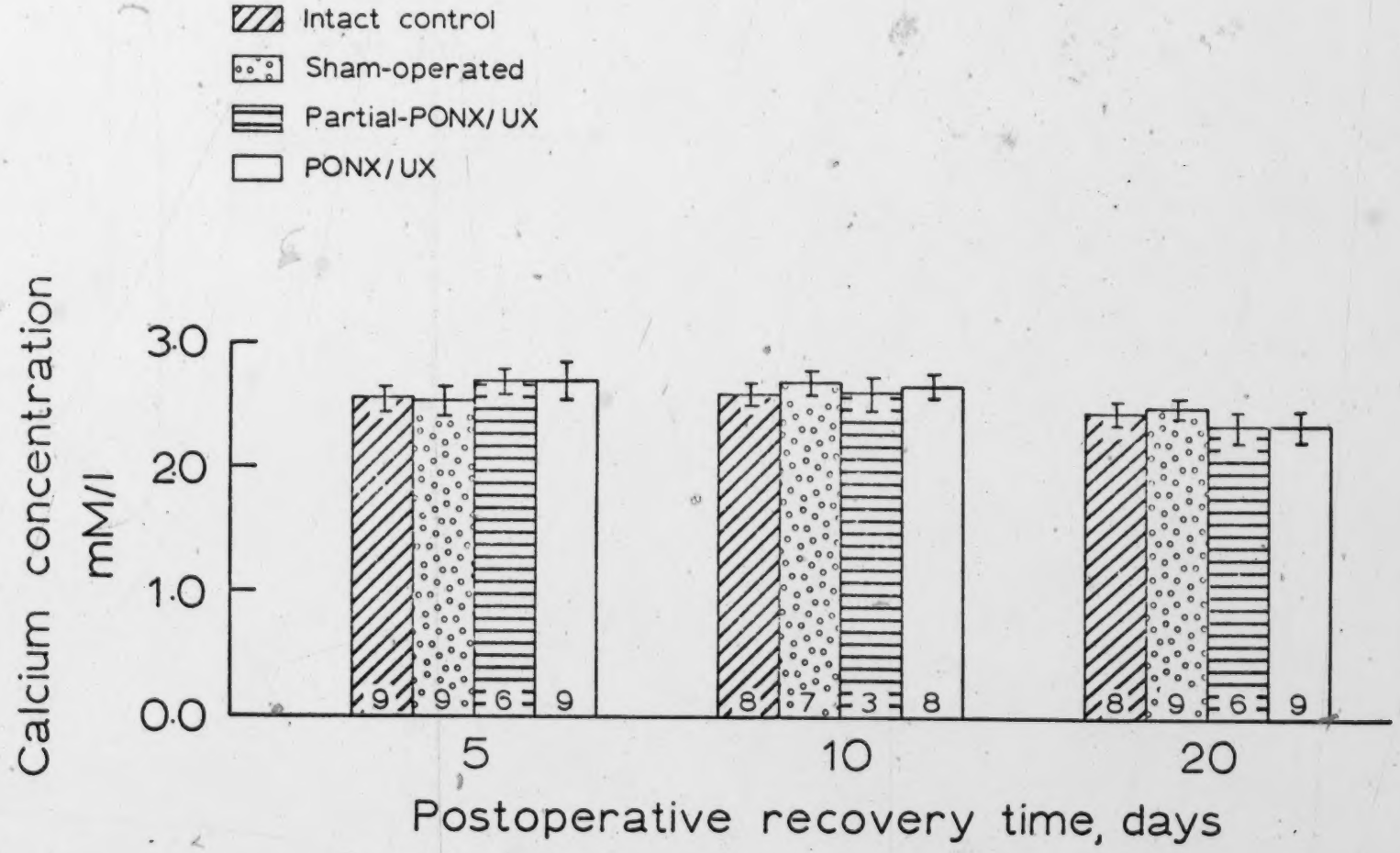

Intact control
Sham-operated
Partial-PONX/UX
PONX/UX
Chloride concentration
mM/l
120
110
100
90
9
9
6
9
7
8
4
7
*
10
10
6
10
5
10
20
Postoperative recovery time, days

The combined operation of the NPO lesion/urophysectomy did not alter the plasma Ca^{++} levels (Figure 34) (Appendix, Table 5). There were no significant differences in the plasma Ca^{++} concentrations between completely lesioned/urophysectomized and partially lesioned/urophysectomized fish at any of the postoperative sampling times, nor were there any differences compared to the sham and intact controls for the same recovery periods. Plasma Ca^{++} concentrations for the sham-operated and intact controls were not different and were in accordance with those obtained in the singly operated animals (Figures 12 and 23). There also were no significant differences between the five, ten and twenty day completely lesioned and urophysectomized fish.

FIGURE 34. The effect of the combined operation of preoptic nucleus lesioning and urophysectomy on the plasma calcium concentration of goldfish. The vertical bars represent ±SEM. The number of individuals in each experimental group is shown at the base of each bar.

Partial - PONX/UX - partially preoptic nucleus lesioned and urophysectomized

PONX/UX - completely preoptic nucleus lesioned and urophysectomized


Intact control
Sham-operated
Partial-PONX/UX
PONX/UX
Calcium concentration
mM/l
3.0
2.0
1.0
0.0
9
9
6
9
8
7
3
8
8
9
6
9
5
10
20
Postoperative recovery time, days

DISCUSSION

Urophysectomy

Plasma and urine electrolyte levels, urinary excretion rates, osmolality and urine flow rates of the sham-operated and intact control fish are in compliance with values obtained for goldfish by other workers (Maetz, 1963; Maetz et al., 1964a; Maetz et al., 1964b; Bourget et al., 1964; Donaldson et al., 1968; Ogawa, 1968; Motais et al., 1969; Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970; Mackay, 1974).

Urophysectomy altered the ability of the goldfish to regulate plasma Na^+ levels. The plasma Na^+ levels, which were significantly depressed at five days postoperatively, returned to control values by the tenth postoperative day. Maetz et al. (1964a) observed intraperitoneal (I.P.) injections of urophysial extracts stimulated branchial Na^+ influx in goldfish causing a net gain of Na^+. Although they found no significant effect on Na^+ efflux, their results were variable (Maetz et al., 1964a). The Na^+ stimulating factor of the urophysis, later to be called Urotensin III (Lederis et al., 1969; Bern and Lederis, 1969; Lederis, 1970c; Berlind, 1973), is apparently a separate

entity from the other urophysial factors (Geshwind, et al., 1968; Lederis, 1969). Thus, the temporarily reduced plasma Na^+ concentration observed in the present study could either be due to an increase in branchial Na^+ efflux or to a reduction in the branchial Na^+ influx due to the absence of this urophysial factor. Furthermore, since urine Na^+ concentration and renal Na^+ excretion was decreased, the decrease in plasma Na^+ was most likely due to Na^+ loss across the gills.

Plasma Cl^- levels were not affected by urophysectomy in the present study. Maetz et al. (1964a) state that in a preliminary experiment I.P. injections of urophysial extracts increased Cl^- influx concurrently with Na^+ influx. The experimental procedure was not described, however. Takasugi and Bern (1962) reported a decrease in serum Cl^- with urophysectomy in the euryhaline teleost Tilapia mossambica maintained in freshwater. The fact that these fish were starved and handled daily for a period of ten days suggests that any difference was probably due to surgical and handling procedures, especially as the decrement was not significantly lower than in the sham-operated group. Because Cl^- excretion was lowered as a result of urophysectomy, and plasma and urine Cl^- concentrations were not altered, there had to have been either an increase in Cl^- efflux or a decrease in Cl^- influx to compensate for the

reduction in renal Cl^- loss.

There were no changes in plasma Ca^{++} levels in the urophysectomized goldfish compared to the sham-operated and intact control fish in this study. Chan (1969) found that urophysectomy did not alter the overall Ca^{++} balance in the European eel. No other studies have been done that indicate that urophysectomy alters Ca^{++} balance in teleosts. Thus, it appears unlikely Ca^{++} is regulated by the urophysis.

Urophysectomy did, however, produce an antidiuresis in the goldfish used in this study. This antidiuresis was, as with the drop in plasma Na^+, transitory. Recovery in urine flow to near control values occurred by ten days postoperatively. Although intraperitoneal and intravenous (I.V.) injections of urophysial extracts elicit an immediate rise in PAH, free water (C_{H_2O}) and inulin clearances, and urine flow in goldfish (Maetz et al., 1964a) and in the freshwater adapted eel (Bern et al., 1967; Chan et al., 1969; Chester Jones et al., 1967, 1969), urophysectomy has, up until the present study, failed to produce any alteration in renal function (Berlind, 1973). The same operation performed on Fundulus kansae and Tilapia mossambica had no effect on urine volume or urinary Na^+ excretion (Imai et al., 1965). However, the urine was collected at more than three weeks postoperatively in Imai's study and the fact that

urine flow had returned to normal values by ten days in the present study could account for the difference between the results of his study and the present one. Chester Jones et al. (1969) also found that urophysectomy did not cause a significant decrease in urine flow or Na^+ excretion in the freshwater adapted eel, Anguilla anguilla. There was, however, a decrease in urine flow from 38.0 ml/kg body weight·day during the initial control period to 31.7 ml/kg b. wt.·day on day seven. The mean value obtained on day five was 28.0 ml/kg b. wt.·day. Also, a significant reduction in GFR (inulin clearance) occurred by day seven between the urophysectomized eels and the sham animals but not between the urophysectomized and intact control group. Although not statistically significant, there was, in the above study, a trend in the urophysectomized eels to have a reduced urine flow up to seven days postoperatively. This reduction in urine volume is similar to the antidiuresis observed in the five day urophysectomized goldfish in the present study. It is possible that in the study by Chester Jones et al. (1969) that diuresis due to handling stress could account for the variability of the data, since their fish were handled daily in order to take measurements.

The reduction in urine flow seen in the present study could be due to either a decrease in GFR or to an

increase in tubular reabsorption of water. It is suggested that the antidiuresis observed in this study was due to a reduction in GFR. This hypothesis is substantiated by Chester Jones et al. (1969) who found a reduction in GFR in eels following urophysectomy and by preliminary studies by Lederis which indicate that the urophysial principles Urotensin I and II affect GFR in these fishes (See Introduction). Recovery, then, could be due to an increase in AVT production from the neurohypophysis resulting in an increase in the GFR.

Urine osmolality and Na^+ and Cl^- levels were not changed significantly by urophysectomy in the present study. Urine Ca^+ concentration was, however, significantly decreased by urophysectomy at five days postoperatively but returned to near control levels by ten days. As a result of the reduction in urine flow in the five day postoperative fish, electrolyte excretion rates were significantly decreased. Because urine flow had increased in the ten day urophysectomized fish, electrolyte excretion rates were similar to control animals.

Imai et al. (1965) found no effect of urophysectomy on urinary Na^+ concentration in F. kansae, or in the ability of T. mossambica to excrete Na^+ after injection of a hypertonic NaCl solution. In the urophysectomized freshwater adapted eel, A. anguilla, Na^+ excretion was not significantly changed although there was an initial rise in Na^+ excretion on the first postoperative day (Chester Jones et

al., 1969). I.V. injections of urophysial extracts, however, caused an immediate rise in urinary Na^+ excretion in freshwater eels (Bern et al., 1967; Chester Jones et al., 1967, 1969). Maetz et al. (1964a) found that the natriuresis as well as the increase in relative Na^+ clearance that occurred with I.P. injections of urophysial extracts in goldfish were followed by a reduction in these parameters to below normal values in the second and third hours after treatment. It was suggested that this reduction corresponded to an increase in tubular reabsorption of Na^+. Because urine Na^+ concentration did not change the reduction in renal Na^+ excretion found in the urophysectomized goldfish in the present study was due to the reduction of urine output.

Urine Cl^- levels with respect to urophysectomy have not been reported in any foregoing work. However, urophysectomy did cause a decrease in Cl^- excretion rates in the goldfish at five days postoperatively. The decrease in Cl^- excretion was, however, proportional to the decrease in urine flow. Maetz et al. (1964a) did not find a change in Cl^- excretion following I.P. injections of urophysial extracts, however, he stated that branchial Cl^- influx was increased (see above). In order for the five day urophysectomized fish to have maintained normal plasma Cl^- concentration, compensatory changes in branchial Cl^- flux

would have had to occur. This could have been either a decrease in influx or an increase in efflux of Cl^-. A decreased Cl^- influx would be expected because urophysial peptides will increase Cl^- influx.

In A. anguilla, Chan (1969) found that urophysectomy caused an immediate calciuresis in the first and second postoperative days. This Ca^{++} loss was subsequently compensated for by renal Ca^{++} retention. Chester Jones et al. (1969) also found an immediate increase in Ca^{++} excretion after urophysectomy in A. anguilla which later declined to very low levels by the fourth postoperative day. The fact that there was Ca^{++} retention in the eels in the above studies lend support for the reduction in urine Ca^{++} concentration and excretion rates in the five day urophysectomized goldfish in the present study. The fact that the plasma Ca^{++} concentration of the fish used in this study did not change suggests there could be renal retention of Ca^{++} in order to maintain normal plasma balance. Conservation of Ca^{++} could be important at this time in order to repair bone tissue that was damaged due to the removal of the urostyle during surgery. Bone loss could create a hypocalcemia, and thus, renal reabsorption of Ca^{++} would be stimulated.

The effects observed following urophysectomy are most likely due to the absence of more than one urophysial

principle. Thus, it is possible that the reduction in urine flow was due to the absence of an entirely different urophysial peptide than that which caused the reduction in plasma Na^+ level. Therefore, one factor, such as Urotensin IV, could be affecting only GFR, reducing total urine output, and at the same time another principle, such as Urotensin III, could be affecting ion transport across the gill.

The temporary effects of urophysectomy are probably not due to the regenerative property of the urophysis, but rather to pituitary (specifically the neurohypophysis) intervention (see General Discussion).

Preoptic Nucleus Lesioning

Lesioning of the NPO caused a decline in the plasma Na^+ levels in Carassius auratus at both five and ten days postoperatively. This hyponatria appears to be of short term duration as normal Na^+ balance returns by twenty days. Electro-cautery of the NPO in freshwater adapted eels, A. anguilla and A. japonica, also caused a reduction in plasma Na^+ in these fishes (Chan, 1969).

It is well documented in the literature that hypophysectomy causes a reduction in plasma Na^+ in a variety of species (A. anguilla, Chan et al., 1968a; Chan, 1969; Chan et al., 1969; Fundulus species, Pickford et al.,

1966; Stanley and Fleming, 1967b; Fleming and Ball, 1972; Pang et al., 1973; Poecilia latipinna, Ball and Ensor, 1967; C. auratus, Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970; Donaldson et al., 1968; Ogawa, 1968). However, the values that Ogawa (1968), Lahlouh and Sawyer (1969) and Lahlouh and Giordan(1970) report for plasma Na^+ levels in hypophysectomized goldfish were much lower than the plasma Na^+ levels in the NPO lesioned goldfish in the present study. Plasma Na^+ in hypophysectomized goldfish, six days postoperatively, dropped to 87.7 mEq/l from the sham and intact control values of 133 and 142 mEq/l respectively (Lahlouh and Giordan, 1970). While plasma levels were 119 mEq/l in the hypophysectomized goldfish at three weeks (Lahlouh and Giordan, 1970). Lesioning of the NPO of the goldfish in the present study resulted in a decrease in plasma Na^+ to 122.5 and 121.5 mM/l at five and ten days respectively with a return to near control values of 136 mM/l at twenty days. These data suggest that, firstly, total removal of pituitary function has a greater effect on plasma Na^+ level (the drop in plasma Na^+ was of greater magnitude) than does just lesioning the NPO. Secondly, there is partial recovery in plasma Na^+ concentration in the hypophysectomized animals by three weeks. The recovery could possibly be due to neurohypophysial peptides, as hypophysectomy is believed not always to eliminate the functioning of the NPO (see Introduction). Thus, Na^+

balance in teleosts is probably normally mediated through a balance in both the adenohypophysial and neurohypophysial systems (see General Discussion).

The phenomena of incomplete ablation of a hypothalmic nucleus not producing identical results as total lesioning has also been found in other studies. Chan (1969) found that, in the European eel, an incomplete lesion of the NPO did not result in the same reduced plasma Na^+ and Ca^{++} levels observed in the totally lesioned fish. Inconsistent results related to electrode deposits of Fe^{++} following D. C. current lesioning of the hypothalmic area have been seen in mammals (Everett et al., 1961; Rabin, 1972). Thus, the increase seen in plasma Na^+ in the partially lesioned fish in the present study could possibly be due to an irritation caused by ionic residue from the electrode.

Lesioning of the NPO had no influence on the plasma Cl^- concentrations of goldfish in this study. Ogawa (1968) and Lahlouh and Sawyer (1969) found a decrease in plasma Cl^- in hypophysectomized goldfish. Pickford and Phillips (1959) found that hypophysectomized killifish, F. heteroclitus died of severe hypochloremia. Furthermore, Pickford et al. (1965) 1966) found the neurohypophysial hormones failed to increase

the Cl^- levels in hypophysectomized F. heteroclitus. Hypophysectomy also reduced plasma Cl^- levels in the eel, A. rostrata (Butler, 1973). Pang et al. (1973), however, did not find a change in Cl^- concentration in the serum of hypophysectomized F. heteroclitus.

There is no direct evidence in the present study that indicates that plasma Cl^- levels are affected by the NPO. However, in order for the ten day lesioned fish to maintain normal Cl^- levels, an increase in Cl^- uptake would have to occur to compensate for the increase in Cl^- excretion for these animals. Because prolactin will stimulate Na^+ uptake (Olivereau and Ball, 1970), there could be a concurrent increase in Cl^- uptake, thus, allowing prolactin to maintain normal plasma Cl^- levels.

Lesioning of the NPO did not change the plasma Ca^{++} levels of the goldfish in the present study. However, Chan (1969) found a decrease in plasma Ca^{++} in both hypophysectomized and NPO lesioned freshwater eels compared to the respective sham-operated fish. Although Chan reported a significant decline in plasma Ca^{++} due to hypophysectomy, he did not indicate whether the decline in plasma Ca^{++} level due to electro-cautery of the NPO was statistically significant. He did not find any significant differences between the sham-operated (forebrain removal) and those with partial removal of the NPO. Reduc-

tion in plasma Ca^{++} due to hypophysectomy has also been observed by Chester Jones et al. (1968) and Chan et al. (1968) in A. anguilla, by Pang (1973a, b) in Fundulus species, and by Ogawa (1968) in Carassius auratus. The results in the present study suggest that the neurohypophysis does not have a role in Ca^{++} regulation. Therefore, the reduction in plasma Ca^{++} observed following hypophysectomy is most probably due to the absence of some factor released from the adenohypophysis (see General Discussion).

In the present study, a marked reduction in urine flow and a concomitant rise in the urine osmolality, and urine Na^{+}, Ca^{++} and Cl^{-} concentrations resulted after lesioning the NPO. There is general agreement that removal of the pituitary gland is followed by a decrease in urine flow rates and an augmentation in urine osmolality and/or urine Na^{+} levels (Chester Jones et al., 1965; Butler, 1966; Stanley and Fleming, 1966, 1967b; Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970; Butler, 1973).

The urine flow rates of the ten day lesioned goldfish in the present study (6.05 ml/kg·hr) are consistent with rates obtained in goldfish three weeks after hypophysectomy (Lahlouh and Sawyer, 1966; 6.04 ml/kg·hr; and Lahlouh and Giordan, 1970, 6.0 ml/kg·hr). Lahlouh and Giordan (1970) obtained urine flow rates of 3.1 ml/kg·hr in hypophysectomized goldfish on the sixth postoperative day which

is comparable to the urine flow rate exhibited by the five day lesioned fish (3.8 ml/kg·hr) in the present study. Thus, it appears that there is a similar time effect on urine flow rates following hypophysectomy and lesioning the NPO. Lahlouh and Sawyer (1969) and Lahlouh and Giordan (1970) found that although prolactin increases urine flow in hypophysectomized fish, it does not cause diuresis in intact goldfish. The neurohypophysial peptide AVT will, however, cause diuresis in both hypophysectomized and intact goldfish (Lahlouh and Giordan, 1970; Sawyer, 1972). Therefore, it is likely that the reduction in urine flow following hypophysectomy is, at least in part, due to the removal of neurohypophysial function. The compensatory changes in urine flow seen by ten days in the NPO lesioned goldfish could be due to prolactin involvement in restoring homeostasis. However, owing to the evidence presented in this paper and the diuretic effect of urophysial extracts found by other workers, it is quite possible that the increase in urine flow rate of the ten day lesioned fish over the five day lesioned fish could be due to changes in urophysial function.

It was observed in the present study that the lesioned fish had larger weight gains and a bloated appearance compared to the sham-operated and intact control fish. These differences in weight gain were not, however, significant. This might be a general hydration due to reduction

in glomerular filtration or an increase in tubular reabsorption of water, as a result of the lesion. Owing to the augmentive effect of AVT on glomerular filtration (Maetz et al., 1964b; Sawyer, 1970, 1972), one would suspect that it could be due to a reduction in GFR. Chan (1969) also observed a rise in body water content and hemodilution when the NPO was cauterized in the freshwater eel, A. anguilla. A gradual increase in body weight which reflected an increase in body water was also observed in the hypophysectomized goldfish (Lahlouh and Giordan, 1970). However, the increase in urine concentration seen in the lesioned fish in this study strongly suggests that there was increased tubular reabsorption of water. This could have been in addition to a decrease in GFR. To determine whether the antidiuresis produced by urophysectomy and NPO lesioning was glomerular in origin, further studies measuring the effects of these operations on inulin and PAH clearance would be necessary.

Urine Na^+ levels of the goldfish used in the present study were significantly increased to 15.1 and 14.7 mM/l in the five and ten day lesioned animals respectively from mean sham-operated and intact control values of 6.9 and 6.4 mM/l respectively. Lahlouh and Sawyer (1969) also found an increase in urine Na^+ concentration from 6.0 mEq/l in the intact goldfish and 8.5 mEq/l in the sham-

operated controls to 15.0 mEq/l in hypophysectomized fish. Although the results in the study by Lahlouh and Sawyer (1969) were not statistically significant, the values they obtained are quantitatively comparable to those presented in the present work. The goldfish in the present study were not subjected to the stress of daily handling which likely made the results less variable than those of Lahlouh and Sawyer (1969). Lahlouh and Giordan (1970) found that the urine Na^+ concentration of goldfish increased progressively from 3.6 mEq/l in intact goldfish to 27.0 mEq/l in the hypophysectomized fish by six days post-hypophysectomy. However, there was no difference in urine Na^+ concentration between the hypophysectomized and intact control fish at three weeks. Urine Na^+ concentrations were also elevated in F. kansae (Stanley and Fleming, 1966, 1967a,b); and in A. rostrata (Butler, 1973) following hypophysectomy. The increase in urinary electrolyte concentration following hypophysectomy and a decrease in urine flow are the only consistent effects of hypophysectomy on renal function in teleosts (Butler, 1973). In the goldfish, prolactin and cortisol were only partially able to restore the urine Na^+ concentrations which were elevated by hypophysectomy, while AVT was able to return urine Na^+ concentration to control values in these fish (Lahlouh and Giordan, 1970). In the same study, neither prolactin or cortisol affected urine Na^+ levels in these fish (Lahlouh and Giordan, 1970).

Therefore, it is possible that the increase in urine Na^+ concentration due to hypophysectomy is due, in part, to the loss of neurohypophysial function. This is supported by the elevated urine Na^+ concentration found in the NPO lesioned fish in the present study.

At ten days postoperatively, the NPO lesioned goldfish used in the present study had Na^+ excretion rates that were significantly higher than control values. Lahlouh and Sawyer (1969) reported that urine output of Na^+ was not altered by hypophysectomy in goldfish three weeks after the operation. This was later substantiated by Lahlouh and Giordan (1970) where Na^+ excretion in goldfish increased until the sixth or seventh day post-hypophysectomy, but returned to the control rate by three weeks. The ratio of renal Na^+ output to plasma Na^+ concentration in the study by Lahlouh and Sawyer (1969) was, however, significantly higher in hypophysectomized goldfish, therefore not eliminating the contribution of the kidney to the loss of plasma Na^+. This ratio was also higher in the NPO lesioned fish in this study. Thus, the resultant hyponatria could be attributed, at least in part, to changes in renal filtration or reabsorption of Na^+. As further support for this interpretation, hypophysectomy has been shown to cause increased Na^+ loss in F. kansae (Stanley and Fleming, 1966, 1967a, b) and an increase of 30-50% in the fraction of fil-

tered Na^+ excreted in A. rostrata (Butler, 1973). Also, hypophysectomized F. kansae showed reduced renal Na^+ reabsorption compared to control fish (Stanley and Fleming, 1966).

Because the NPO lesioned fish were losing Na^+, compared to the sham and intact control groups, regulatory adjustment in Na^+ uptake would have had to occur in order for the twenty day lesioned fish to have normal plasma Na^+ levels. Prolactin is able to increase branchial (Dharamamba et al.) and intestinal (Bern, et al., 1974) Na^+ influx and could, therefore, have elevated the plasma Na^+ level. Some regulation may have come from the urophysis, as Urotensin III is also believed to increase Na^+ uptake.

Goldfish urine Cl^- concentrations in the present study were elevated by lesioning the NPO. This was possibly due to an increase in tubular reabsorption of water. Cl^- excretion had not changed by the fifth postoperative day but was significantly increased by the tenth postoperative day. Changes in urinary Cl^- excretion between the lesioned fish at the two postoperative times are a result of changes in urine flow, as there was no difference in either plasma or urine Cl^- levels between the two lesioned groups. Maetz et al. (1964b) found that urine Cl^- concentrations in goldfish remained unchanged with I.P. injections of AVT. These authors did find a transient increase in urinary Cl^- excretion which was proportional to the diuresis produced by the AVT.

Butler (1973) found that the urine concentration of Cl^-, Cl^- excretion rate, and the rate of Cl^- excreted relative to the amount of Cl^- filtered were significantly elevated by hypophysectomy in the freshwater adapted eel, A. rostrata. Because urine Cl^- levels and excretion rate (in the ten day fish) were elevated following lesioning of the NPO, a change in branchial Cl^- influx must have occurred for plasma Cl^- to remain at normal values.

Urinary Ca^{++} concentrations were also elevated by NPO lesioning. Again, this was probably due to reabsorption of water in the distil tubule. Hypophysectomy also resulted in an elevated urine Ca^{++} concentration in F. kansae (Stanley and Fleming, 1967b). Ca^{++} excretion was significantly lowered in the goldfish five days after lesioning due to the reduced urine flow. At ten days, the elevation in urine concentration counterbalanced the reduction in urine flow in the lesioned fish, resulting in a Ca^{++} excretion rate that was comparable to that of the control animals.

Because neither the plasma Ca^{++} concentrations or the Ca^{++} excretion rate were altered following the lesioning of the NPO of the ten day fish, the increase in urine Ca^{++} concentration was probably due to increased tubular reabsorption of water, causing a concentration of the urine. Therefore, the ten day lesioned fish were in

Ca^{++} balance. The difference between the five and ten day lesioned fish was an increase in urine flow in the latter group. This increase in urine flow could have been due to an increase in GFR mediated by either a urophysial principle or by prolactin, or due to a decrease in tubular reabsorption of water.

To support the hypothesis that changes in prolactin secretion could have compensated for the reduction in Ca^{++} excretion in the five day lesioned fish, hypophysectomized F. kansae had greater renal loss of Ca^{++} than either sham or normal controls two weeks after the operation (Stanley and Fleming, 1967b).

Preoptic Nucleus Lesion/Urophysectomy

The plasma Na^{+} concentrations of the completely and partially lesioned/urophysectomized fish at five days postoperatively, and of the completely lesioned/urophysectomized fish at ten days postoperatively were significantly lower than in the sham and intact control groups. Because the partially lesioned fish at ten days postoperatively did not have reduced plasma Na^{+} levels (Figure 21), the reduction in the partially lesioned/urophysectomized five day fish was most likely due to loss of urophysial function. This confirms results of the effects of urophysectomy along on plasma Na^{+} levels.

There was no additive effect of the combined operations, since the Na^+ levels found in the doubly operated fish were the same as in the single operated (urophysectomized or NPO lesioned) fish. Lacanilao (1972a) found that the effects of submaximal doses of urophysial extracts (4 µg/ml) and oxytocin (100 µU/ml) on water loss in isolated toad bladders were synergistic, but at maximal doses (20 µg and 500 µU respectively) their combined effect was no greater than either alone. This offers an explanation as to why the plasma Na^+ concentration in the doubly operated fish was not lower than singly operated fish and suggests that similar sites (i.e., the gill) were affected by the operations. The fact that isotocin will stimulate branchial Na^+ influx (Maetz et al., 1964b) as will Urotensin III (Maetz et al., 1964a) supports the above explanation.

The reduction in plasma Na^+ in the present study does not appear to be chronic as normal Na^+ balance (no differences between experimental and control groups) was attained by the twentieth postoperative day. As regeneration of the urophysis is probably not a factor in this experiment (see General Discussion), the recovery of the plasma Na^+ levels could be due to changes in the endogenous prolactin secretion (see General Discussion).

Plasma Cl^- and Ca^{++} levels were not affected by the combination of the operations. This supports the findings

of the present study where no effects on plasma Cl^- and Ca^{++} levels were found following urophysectomy or NPO lesioning.

General Discussion

There has been considerable evidence in recent years that the corpuscles of Stannius and pituitary gland control Ca^{++} metabolism in teleosts (Chan, 1968; Chan et al., 1968a; Chan and Chester Jones, 1968; Chan et al., 1969; Pang, 1971, 1973; Pang et al., 1973). Although Chan et al. (1968b) and Chan (1969) have suggested that preparations of calcitonin from the ultimobranchial body have a hypocalcemic effect in A. anguilla and A. japonica, subsequent workers have failed to confirm this in other species (see Pang, 1973). The current hypothesis (Pang, 1973; Pang et al., 1973) concerning the endocrine control of calcium metabolism postulates that the pituitary, mediated by prolactin, has a distinct hypercalcemic function which is manifested in low Ca^{++} environments regardless of the Na^+ level and osmotic conditions of the environment. On the other hand, the corpuscles of Stannius function in a hypocalcemic manner to allow regulation in environments high in Ca^{++} (Pang, 1973; Pang et al., 1973). Pang et al. (1973) found that neurohypophysial hormones would not raise plasma Ca^{++} in hypophysectomized fish in a hypocalcemic environment, but prolactin could.

Urine Ca^{++} concentration was, however, elevated following NPO lesioning in the present study. This could have been due to an increase in tubular reabsorption of water. On the other hand, the decrease in urine Ca^{++} concentration that occurred following urophysectomy was due to renal retention of Ca^{++}. The changes observed in Ca^{++} excretion thus, were probably not the primary effects of these operations. Renal retention of Ca^{++} in the urophysectomized fish could have been caused by an increase in prolactin.

Although prolactin has been hypothesized as being the major hypophysial hormone involved with maintenance of Na^{+} homeostasis in hypophysectomized teleosts (Pickford and Phillips, 1959; Pickford et al., 1966; Pickford and Pang, 1966; Fleming and Ball, 1972; Ball and Ensor, 1967), other workers have found that injections of prolactin are not able to entirely prevent the fall in plasma Na^{+} or osmolality in hypophysectomized freshwater fish (Dharamambo et al., 1967; Donaldson et al., 1968; Chan, 1968; Chan et al., 1968a; Lahlouh and Sawyer (1969). Prolactin also is not diuretic in the intact goldfish (Lahlouh and Giordan, 1970). Thus, it is possible that other hypophysial hormones such as AVT, isotocin or ACTH could account for the Na^{+} deficit in hypophysectomized fish in the above studies. As ACTH has no effect on plasma Na^{+} in hypophysectomized

goldfish (Lahlouh and Giordan, 1970), it is possible to speculate that the neurohypophysial hormones also affect Na^+ regulation. This is supported indirectly by the fact that the drop in plasma Na^+ in the NPO lesioned fish is not due to a change in prolactin secretion because the preoptic area has been shown to have no influence on the plasma or pituitary prolactin levels (Peter and McKeown, 1974). However, the return to normal plasma Na^+ levels could be due to compensatory adjustments in prolactin secretion. Prolactin increases branchial permeability (Lahlouh and Giordan, 1970) and stimulates branchial Na^+ uptake (Stanley and Fleming, 1967a; Fleming and Ball, 1972); therefore; prolactin could have restored the plasma Na^+ levels in the NPO lesioned fish.

The results from this study indicate that urophysectomy also has a hyponatremic effect on the goldfish, which is similar to that seen by ablation of the NPO. Recovery from this hyponatria can be accounted for by pituitary control of Na^+ balance either through a functional NPO or through prolactin secretion.

Though regeneration of the urophysis has been reported in some species (Fridberg et al., 1966), this does not begin until about two weeks after urophysectomy. At this stage, in the species investigated, there was no

stainable neurosecretory material nor was there any contact between the neurosecretory axons and capillaries. There were only a few cells that showed rudimentary signs of becoming neurosecretory cells. By 22 days, however, these subependymal cells could be identified as Dahlgren cells. However, there was still no contact between the neurosecretory axons and the capillaries. It was not until 5-6 months that structural regeneration of the neurohemal organ was complete (Fridberg et al., 1966). Regeneration is, therefore, probably not an influential factor in the present study. Both the neurohypophysial peptides, Isotocin and AVT, will stimulate Na^+ influx across the gills (Maetz, 1963; Maetz et al., 1964b) as will Urotensin III (Maetz et al., 1964a). Because of the similarity of the effects of urophysectomy and NPO lesioning on plasma Na^+ levels in the present study the recovery seen in the plasma Na^+ levels of the ten day urophysectomized fish was probably due to an increase in neurohypophysial function.

Maetz et al. (1964a) found that, in goldfish, the response of Na^+ movement to I.P. injections of urophysial extracts was more accentuated on the gill than on the kidney. Conversely, Maetz et al. (1964b) found that the neurohypophysial peptides affected the kidney more than the gills. These observations support the findings in the present data that renal Na^+ loss was greater in the NPO

lesioned fish than in the urophysectomized fish.

In the present study, there were no changes in urine Na^+ concentration following urophysectomy. Therefore, the hyponatria caused by urophysectomy could be due to branchial Na^+ loss, indicating that the gill is an important target organ for the urophysial principles.

Recovery of plasma Na^+ in the doubly operated fish was most likely due to changes in prolactin secretion, because the NPO was destroyed and, therefore, unable to contribute in Na^+ regulation. In addition, the regenerating urophysis is not believed to be functional in this time period.

As urophysectomy did not alter urine electrolyte concentrations, the decrease in electrolyte excretion was due to a reduction in urine output. This antidiuresis could be due to the absence of such urophysial principles as the hydrosmotic and/or the trout bladder contracting urophysial factors, Urotensin IV and II, respectively (see Introduction). The hydrosmotic factor, Urotensin IV, has chromatographic properties and a pharmacological profile similar to that of AVT (Lacanilao, 1972a, b). If such is the case, then it is possible that the antidiuretic effect of lesioning the NPO and urophysectomy could be due to the absence of AVT. The transitory decrease in urine flow

as a result of urophysectomy can be explained by hypothalmic control resulting in an increased secretion of AVT from the neurohypophysial tissue. This hypothesis is supported by the observation by Takasugi and Bern (1962) that urophysectomy resulted in a hypertrophy of the NPO. The hydrated state of the NPO lesioned fish (see above) could have been caused by increased renal tubular reabsorption of water, thus explaining the increase in urine concentration. This hydration was not observed in the urophysectomized fish.

This study has shown that there is a time sequence in the events following lesioning of the preoptic nucleus and urophysectomy. In both cases urine flow was higher at ten days postoperatively than at five days. This increase in urine flow resulted in increased urine electrolyte loss in the ten day lesioned group. Urine flow and electrolyte excretion could possibly return to near normal levels by twenty days as other pituitary factors became involved in osmotic and ionic regulation. This was suggested (see above) to be the case in the return of plasma Na^+ to control levels by twenty days post-lesion. The changes in urine flow and urine electrolyte excretion rates over time in hypophysectomized F. kansae (Stanley and Fleming, 1966) and in the hypophysectomized goldfish (Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970) lend support to this hypothesis.

The responses seen following the injection of urophysial and neurohypophysial extracts are analogous to the effects of artificially lowering concentrations of plasma electrolytes (Bourget et al., 1964). These facts, combined with the observations of Lahlouh and Giordan (1970) that AVT caused a decrease in branchial water permeability and is strongly diuretic, suggests that the urophysis and the neurohypophysis respond to an internal hypoosmotic stimulus, thus allowing a freshwater teleost to eliminate excess water. Lahlouh and Giordan (1970) suggest that normal water balance in the teleost is maintained by the opposing actions of prolactin and AVT. Prolactin, although it will cause diuresis, is believed mainly to increase the permeability of the skin and gills to water, thus resulting in an osmotic influx of water (Stanley and Fleming, 1967a; Lahlouh and Sawyer, 1969; Lahlouh and Giordan, 1970). AVT would then be released in response to the waterload created by prolactin to restore normal hydration of the tissues.

Further physiological studies are required in order to determine what physiological role the neurohypophysis and the urophysis play in ionic and osmotic regulation. In addition to the present study, the effects of urophysectomy and NPO lesioning on branchial salt and water flux in conjunction with inulin clearance studies would resolve whether it was renal or branchial compensation that occurred due to these operations.

The simultaneous lesioning of the hypothalmic control centers for the neurohypophysial hormones and prolactin, and the comparison with the effects observed by ablation of either one of these centers alone would define the roles that these peptides have in osmotic and ionic regulation.

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APPENDIX

Table 1. The effect of urophysectomy on the Concentration of Na^+, Cl^- and Ca^{++} in goldfish plasma at five, ten and twenty postoperative time periods

Experimental Group	Postoperative Recovery Time (Days)	Na^+	Cl^-	Ca^{++}
			mM/l	
Intact control	5	134.4 ± 2.6 (9)	117.7 ± 1.1 (9)	2.3 ± 0.06 (9)
	10	133.1 ± 2.1 (10)	116.9 ± 2.1 (10)	2.6 ± 0.11 (10)
	20	132.1 ± 1.7 (9)	119.1 ± 4.5 (9)	2.4 ± 0.09 (9)
Sham-operated	5	134.8 ± 1.5 (10)	118.0 ± 2.5 (8)	2.3 ± 0.09 (9)
	10	132.5 ± 1.7 (10)	115.8 ± 2.3 (9)	2.6 ± 0.19 (9)
	20	131.3 ± 0.9 (10)	113.0 ± 2.6 (11)	2.7 ± 0.12 (8)
Urophysectomized	5	117.7 ± 2.9** (9)	115.9 ± 1.8 (9)	2.4 ± 0.16 (9)
	10	134.5 ± 1.8 (10)	115.1 ± 2.9 (9)	2.6 ± 0.19 (10)
	20	135.0 ± 2.0 (10)	114.6 ± 2.3 (10)	2.6 ± 0.17 (10)

Values are mean ± SEM. The number of fish in each group (N) is given in brackets.

** $p < 0.01$

Table 2. The effect of urophysectomy on the urine flow, osmolality, electrolyte concentration and excretion rates of goldfish at five and ten days postoperatively

Experimental Group	Postoperative Recovery Time (Days)	Urine Flow (ml/kg·hr)	Urine Osmolality (mOsm)	Urine Electrolyte Concentration Na^+ (mM/l)	Cl^- (mM/l)	Ca^{++}	Excretion Rate Na^+ (uM/kg·hr)	Cl^- (uM/kg·hr)	Ca^{++}
Intact Control	5	9.5 ± .45 (15)	29.7 ± .95 (9)	6.9 ± .95 (9)	3.7 ± .2 (9)	0.62 ± .02 (9)	65.4 ± 2.6 (8)	34.7 ± 1.6 (8)	5.7 ± .26 (8)
	10	9.3 ± 2.4 (18)	28.7 ± .8 (11)	6.6 ± .18 (11)	3.7 ± .26 (11)	0.61 ± .06 (11)	60.6 ± 1.7 (11)	33.3 ± 2 (11)	5.7 ± .29 (11)
Sham-operated	5	9.6 ± .41 (16)	28.7 ± 1.3 (11)	6.6 ± .3 (11)	3.8 ± .24 (11)	0.63 ± .04 (11)	62.6 ± 3.2 (11)	35.8 ± 1.8 (11)	6.0 ± 1.46 (11)
	10	9.2 ± .23 (14)	28.3 ± 1.0 (9)	6.5 ± .2 (9)	3.3 ± .16 (9)	0.57 ± .02 (9)	60.2 ± 2.2 (9)	30.9 ± 2.2 (9)	5.3 ± .17 (9)
Urophysectomized	5	5.95 ± .22** (10)	27.9 ± .37 (10)	5.6 ± .16 (10)	3.7 ± .2 (10)	.46 ± .02 (10)	31.5 ± 1.3* (9)	20.3 ± 1.3* (9)	2.6 ± .13* (9)
	10	8.36 ± .21 (15)	28.6 ± 1.0 (11)	6.3 ± .46 (11)	3.9 .28 (11)	.59 ± .02 (11)	54.4 ± 4.8 (11)	53.3 ± 3.3 (11)	5.1 ± .28 (11)

Values are mean ± SEM. The number of fish in each group (N) is given in brackets.

* $p < 0.05$, ** $p < 0.01$

Table 3. The effect of lesioning the preoptic nucleus of goldfish on plasma Na^+, Cl^- and Ca^{++} concentrations at five, ten and twenty postoperative time periods

Experimental Group	Postoperative Recovery Time (Days)	Na^+	Cl^- mM/l	Ca^{++}
Intact Control	5	133.4 ± 1.2 (10)	119.2 ± 2.1 (9)	2.3 ± 0.09 (10)
	10	132.4 ± 1.1 (10)	116.9 ± 3.2 (8)	2.6 ± 0.4 (10)
	20	139.1 ± 1.4	111.8 ± 1.9 (7)	2.5 ± 0.12 (9)
Sham-operated	5	133.8 ± 0.9 (10)	116.8 ± 3.0 (7)	2.4 ± .09 (10)
	10	134.6 ± 0.6 (8)	112.8 ± 2.7 (10)	2.5 ± .09 (9)
	20	139.1 ± 0.98	115.8 ± 3.1 (10)	2.4 ± .12 (10)
Preoptic Nucleus Lesioned	5	122.6 ± 1.7* (9)	118.0 ± 1.5 (9)	2.3 ± 0.14 (9)
	10	121.5 ± 1.9* (10)	118.98 ± 3.8 (11)	2.5 ± 0.08 (11)
	20	136.4 ± 2.8 (11)	111.9 ± 2.8 (10)	2.8 ± 0.22 (10)
Partially Preoptic Nucleus Lesioned	5	135.3 ± 1.4 (7)	114.9 ± 1.5 (7)	2.3 ± 0.11 (7)
	10	148.0 ± 2.8 (5)	118.8 ± 4.8 (5)	2.7 ± .28 (5)
	20	141.1 ± 2.1 (5)	120.2 ± 5.4 (5)	2.4 ± .15 (6)

Values are mean ± SEM. The number of fish in each group (N) is given in brackets.

* $p < 0.05$

Table 4. The effect of lesioning the preoptic nucleus on urine flow, osmolality, electrolyte concentration and excretion rates of the goldfish at five and ten days postoperatively

Experimental Group	Postoperative Recovery Time (Days)	Urine Flow (ml/kg·hr)	Urine Osmolality (mOsm)	Urine Electrolyte Concentration (mM/l)			Excretion Rate		
				Na^+	Cl^-	Ca^{++}	Na^+	Cl^-	Ca^{++}
Intact Control	5	9.3 ± .32 (9)	28.9 ± 1.2 (9)	6.6 ± .29 (9)	3.8 ± .12 (9)	0.63 ± .03 (9)	62.6 ± 3.8 (9)	36.1 ± 1.6 (9)	5.9 ± .49 (9)
	10	9.17 ± .24 (12)	29.1 ± .9 (9)	6.3 ± .28 (9)	3.6 ± .3 (9)	0.56 ± .03 (9)	58.5 ± 2.1 (9)	32.7 ± 2 (9)	5.3 ± .22 (9)
Sham-operated	5	9.8 ± .5 (14)	27.9 ± .7 (11)	6.8 ± .17 (11)	3.3 ± .13 (10)	0.59 ± .02 (11)	63.1 ± .35 (11)	31.4 ± 1.9 (11)	5.5 ± .31 (10)
	10	9.5 ± .23 (12)	29.1 ± 1.2 (10)	7.2 ± .32 (10)	3.6 ± .11 (10)	0.54 ± .03 (10)	28.9 ± 4. (10)	33.4 ± 1.9 (10)	4.9 ± .39 (10)
Preoptic Nucleus Lesioned	5	3.8 ± .23** (11)	48.9 ± 5.6** (9)	15.1 ± 1.5* (9)	7.5 ± .6** (9)	0.74 ± .02* (9)	54.7 ± 5.7 (8)	27.2 ± 2.1 (8)	3.1 ± 3.7 (8)
	10	6.1 ± .17** (11)	43.7 ± 1.9** (9)	14.7 ± .7** (9)	7.5 ± .3** (9)	0.7 ± .02* (10)	89.9 ± 4.7** (9)	46.3 ± 2.7* (9)	4.3 ± .17 (9)

Values are mean ± SEM. The number of fish in each group (N) is given in brackets.

* $p < 0.05$, ** $p < 0.01$

Table 5. The effect of combined preoptic nucleus lesioning and urophysectomy on plasma Na^+, Cl^- and Ca^{++} concentrations in goldfish at five, ten and twenty postoperative time periods

Experimental Group	Postoperative Recovery Time (Days)	Na^+	Cl^- (mM/l)	Ca^{++}
Intact Control	5	134.9 ± 1.6 (10)	115.4 ± 2.0 (9)	2.6 ± 0.1 (9)
	10	129.7 ± 1.6* (7)	111.1 ± 2.5 (7)	2.6 ± .06 (7)
	20	134.8 ± 2.2** (9)	118.9 ± 1.3 (10)	2.5 ± .12 (9)
Sham-operated	5	133.5 ± 2.8 (8)	111.99 ± 2.6 (9)	2.6 ± .08 (9)
	10	125.6 ± 1.4 (8)	105.9 ± 2.6 (8)	2.7 ± .1 (8)
	20	136.6 ± 1.9 (8)	114.1 ± 1.3 (10)	2.5 ± .09 (8)
Preoptic Nucleus Lesion/ Urophysectomy	5	119.5 ± 1.0 (9)	109.6 ± 1.7 (9)	2.7 ± .15 (9)
	10	108.8 ± 3.3** (8)	95.8 ± 2.9 (7)	2.7 ± .09 (8)
	20	132.1 ± 1.7 (10)	115.1 ± 2.5	2.4 ± 0.09 (9)
Partial Preoptic Nucleus Lesion/Urophysectomy	5	120. ± 4.2 (6)	113.6 ± 2.4 (6)	2.3 ± .14 (6)
	10	136. ± 1.5 (3)	113.7 ± 1.5 (4)	2.6 ± .08 (3)
	20	134.8 ± 2.8 (6)	112.9 ± 2.1 (6)	2.3 ± .11 (6)

Values are mean ± SEM. The number of fish in each group (N) is given in brackets.
* $p < 0.05$, ** $p < 0.01$